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Research Article

The Effect of Crude Khat Extract on the Color Stability of a Nano-Composite Resin Material. An *In Vitro* Study

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Abstract

Background: The use of resin composite for restoring teeth has been increased greatly in recent years. Manufacturers are claiming that the nanofilled composite technology has been improved to be similar to the ceramics in shade selection and color stability. In Yemen, khat (Catha) chewing habits may affect the color of esthetic restoration, and it is always accompanied by drinking water and cola.

Aim: The aim of this study is to evaluate the effect of crude khat extract on color stability of Nano-composite compared to microhybrid composite resin.

Materials and Methods: A total of fifty-four specimens were prepared from each of the composite resin material in the form of circular discs. The prepared specimens were representing the two main groups (I and II), these were subdivided randomly into six equal sub-groups (a-f). Staining solutions were (crude khat extract, cola-mineral water and artificial saliva as a control group). The specimens were immersed in the corresponding staining solutions for four hours daily and kept in an incubator at 37°C for a total of 180 hours. The solutions were changed every 24 hours. Colorimeter was used for color change testing, then collected data was analyzed using SPSS version 20.

Results: Microhybrid composite resin showed higher degree of discoloration than nanofilled composite resin.

Conclusion: Khat and drinking solutions were significant factors that affected the color stability of composite restorative materials, nanofilled composite showed the least color change.

Keywords: *Nano-composite Resin; Color Stability; Tooth Discoloration; Catha; Beverages*

Introduction

Discoloration of the composite resin restorative materials is a very important property influencing its clinical use. Unfortunately, color change still one of the reasons for its replacement, which occurs due to exposure to foods, drinks, chewing habits, and overall mouth environment. Even though the improvement in the quality of composite resin restorations due to advances in dental materials technologies, color instability of these materials still challenging disadvantage in long term clinical trials [1,2]. Assessment of the rate of the success or failure of composite resin restoration in clinical practice, can be related to color stability and discoloration assessment that is commonly used as an outcome measure tools [3].

Generally, color change of restorative material described as an extrinsic and intrinsic discoloration. Extrinsic discoloration caused by adhesion of dental plaque and other surface stains, leading to degradation or slight penetration of the superficial layer by the staining agents. While, intrinsic discoloration, is owing to structural properties and physicochemical reactions within the deeper layers of the restoration [4]. Extrinsic discoloration mainly caused by colorants, which contained in drinks and foods, smoking, and poor oral hygiene. It is also associated with incomplete polymerization and inefficient polishing technique [5]. Furthermore, the matrix composition of the resin materials can affect water sorption, solubility, hydrophilicity and microstructure of the composite resin materials which play a role in restoration discoloration. Both adsorption and absorption of extrinsic agents on the resin restorations leads to color change and affects esthetic outcomes. However, dealing discoloration caused by colorant adsorption and that of colorants absorption is completely different. Good polishing and improved oral hygiene can remove adsorbed colorants, while replacement of the restoration might be needed to manage the cases of absorbed colorants [6,7]. Most studies of color stability involve immersion of the composite discs in staining media at 37°C for a period of time and measure color changes after rinsing of the discs [1,7-9]. This can measure the colorants adsorbed on the surface of the restoration materials, but does not measure discoloration caused by absorption. From clinical viewpoints, these techniques of studies are of limited values as the discoloration caused by adsorption and that caused by absorption are different regarding the removal procedures. An *in vitro* study have reported that various drinks and food ingredients leads to staining of the restoration surface [10]. If these stains are superficial, it can be cleared using suitable polishing techniques, otherwise the management is to refill using a new restoration [11]. Moreover, surface smoothness and its susceptibility to staining was found to be related to the material particles size and finishing and polishing procedures [12]. Rough surface that associated with wearing and degradation, it also compromise the gloss and increase the liability to extrinsic staining [13]. Water sorption might make the resin matrix to be softer, reduce its stain resistance, and degrade its ingredients [7]. In Yemen, one of the most common habits that may affect color of esthetic restorations is khat chewing. Khat is the name generally used for Catha Edulis forssk, a dicotyledonous evergreen shrub [14]. Long term use of khat chewing leads to negative impact on liver function, permanent tooth discoloration, and susceptibility to ulceration of mucosa [15]. The effect of khat chewing on discoloration of esthetic restoration has received little attention [15]. Therefore, this study was performed to evaluate the effect of khat extract with some beverages on color stability of hybrid and Nano-composite restorative materials.

Materials and Methods

Two commercially available composite resin products of different types of filler particles were used in this study. The commercial brand name, composition and manufacturer of the materials used are; Composan LCM microhybrid resin, which composed of 60% inorganic fillers size 0.5 - 2 μm (Promedica, Germany), and Composan bio-esthetic nanofill composite resin, which composed of 78% inorganic filler size 0.7 μm (Promedica, Germany), with the shade of both materials was A2.

Samples preparation

One hundred and eight circular disc samples were prepared with 2 mm thickness and 8 mm in diameter, fifty-four discs were assigned for each study material. Resin composite discs were prepared by condensing them into a Teflon ring mold. After packing of the material into the mold, a celluloid strip was pressed onto the surface of the mold, with two glass slides in order to obtain a flat surface without bubbles, and excess material was eliminated by pressing the glass slide onto the mold with 200 grams weight, producing smooth flat surface. The weight was removed after one minute, and specimen was polymerized for 40 seconds according to the manufacturer's directions, using Radical light emitting-diodes with light intensity of 470 mw/cm^2. The distance between the light source and the specimen was standardized, by the use a 1 mm glass slide thickness. The specimens were taken out of the mold after light curing and were stored in distilled water at 37°C for 24 hours for rehydration and polymerization. The specimens were finished and polished using Sof-Lex disc polishing system (Hawe Micro-Disc, Switzerland), starting by coarse, medium, fine and then superfine discs using low–speed hand piece for 20 seconds, then rinsed with distilled water for 20 seconds, and dried with paper towels, after that, immersed in distilled water for 24 hours at 37°C. The specimens were randomly divided into six equal sub-groups, (a-f) comprising 9 discs each for each study material, representing the different types of staining media used in this study as shown in table 1.

Group Composite Material	Sub-group (a-f)	Staining solutions	Sample size for each staining medias
GI Microhybrid composite material Composan LCM N=54		a) PBS b) MW c) K d) CC e) KMW f) KCC	9
GII Nano-composite Composan bio-esthetic N=54		a) PBS b) MW c) K d) CC e) KMW f) KCC	9

Table 1: *The experimental design of the study.*
Staining medias are (a) phosphate buffer saline (PBS) control group, (b) mineral water (MW), (c) khat extract (K), (d) coca cola (CC), (e) khat extract with mineral water (KMW), and (f) khat extract with coca cola (KCC).

Khat extract preparation

Khat extract was prepared at Molecular Biology Research Lab, University of Science and Technology Hospital, Sana'a, Yemen from fresh khat leaves and twigs, that is cultivated in Yemen. Leaves and twigs were chopped manually, using manual chopper to be coarse in consistency and then were backed in plastic cup.

Based on the minimum quantity of khat chewed and normal stimulated salivary rate,100 grams of clean khat which was rinsed with running water and chopped to coarse consistency, fresh Khat leaves and twigs had been extracted, with 240 ml phosphate buffered saline at 37°C for 4 hours. Chopped khat were measured by electronic scale in a plastic cup and placed in the shaking machine over 4 hours at 37°C, with 200 rpm shaking power. The khat extracts were filtered using filter paper, and stored at 4°C.

Phosphate buffer saline (PBS) preparation

Two tablets of PBS were mixed with 1000 ml distilled water and the mixing solution stored at 4°C, in an incubator.

Aqueous khat extract preparation

Aqueous khat extract with mineral water was prepared by adding 100 grams of khat extract to 100 ml of mineral water in a ratio of 1:1, while aqueous khat extract with cola was prepared by adding 100 grams of chopped khat, to 50 ml of cola in a ratio of 1:0.5.

Immersion of specimens in staining media

To mimic the daily consumption of the beverages in association with khat chewing, each sample of the composite resin specimens (n = 9 per sub-group) was immersed in staining medias (PBS, Aqueous Khat Extract [K], Mineral Water [MW], Coca Cola [CC], Aqueous Khat Extract with Mineral [KMW] and Aqueous Khat Extract with Coca Cola [KCC]), for four hours per day, for a period of 180 hours, that equals 45 days. After immersing in each media, the samples were rinsed with distilled water, and then were stored in distilled water at 37°C for in an incubator for 20 hours. This procedure was repeated for 45 days, and each staining media was renewed every day.

Color evaluation

The base line color values (L*, a*, b*) of all samples, in each group were measured using the Colorimeter (Portable Color Difference Meter TCD 100 Beijing- China) based on CIE L*, a*, b* color coordinate system which allows determination of the three dimensions. L* represents lightness and darkness value, and a* value that measures redness (positive a*) or greenness (negative a*), while b* value measures the yellowness (positive b*) and blueness (negative b*). Color alteration values (ΔE) was calculated by applying the formula $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$, to compare the differences between base line color with the color values after 180 hours of immersion in different media.

Statistical analysis

Data were collected, tabulated and statistically analyzed by SPSS software Windows version 20. Descriptive statistics were shown as Mean ± St.d. One-way analysis of variance (One-Way ANOVA) was used to compare the color change values, among the tested resin composite materials, with six different immersion solutions (PBS, MW, CC, K, KMW, KC), between two immersion times (0 and 180 hrs.). For comparison with control group, and with the other groups, Post Hoc-Bonferroni test was used at a significant level of .05.

Results

All color values revealed changes after immersion in different staining media. L* values (brightness/darkness, where 0 = black and 100 = white) showed that, color change was perceptible after immersion in K extract (57.7), KMW (57.4) and KCC (58.4) staining media, while the color change was not perceptible in samples immersed in PBS (61.6), and in MW (61.8) staining media. Decreased L* values denoted that the color became darker and less bright. In microhybrid composite samples, the maximum change was found in KMW recording L* value of (57.4), while in Nano-composite, the samples immersed in K revealed the highest change L*value (52.9). In case of a* value (change along red-green axis), the value was positive in microhybrid after exposure to all staining media, indicating a shift toward red color. On the other hand, Nano-composite resin samples revealed positive values for PBS, K, KMW and KCC, while negative values were seen for CC and MW, which means shift toward green color. The b* values (change along yellow-blue axis) was found to be positive for both materials in all staining media, indicating shift towards yellow axis (Table 2).

Staining media	Color values	L* Mean color changes		a* Mean color changes		b* Mean color changes	
		Microhybrid	Nanofill	Microhybrid	Nanofill	Microhybrid	Nanofill
PBS		61.6	62.7	0.7	0.08	8.8	7.1
MW		61.8	63.7	2.1	-0.5	10.3	8.9
CC		62.1	63.1	2.1	-3.1	10.9	9.1
K		57.7	52.9	1.7	1.3	13	9.6
KMW		57.4	58.9	1.4	0.25	16.68	10.1
KCC		58.7	60.7	3.1	1.2	12.2	10.1

Table 2: *Mean color values of both studied materials.*

Comparing the two groups of tested materials within the sub-groups staining media using ANOVA test, revealed significant change (p = 0.001), between microhybrid and Nano-composite in sub-groups immersed in K, KMW and KCC, while no significant change (p = 1.000), in sub-groups immersed in PBS, MW and CC.

Further analysis was performed with Post Hoc test with multiple-range Bonferroni test, to reveal the staining differences between each staining medium versus control group (PBS), in both tested materials, as shown in table 3. showed that statistically significant difference (P < 0.001), was found among sub-groups (K, KMW and KCC) versus the control group (PBS), for both tested materials. While no statistically significant difference was found p = 1.000, between mineral water and cola alone versus control group.

As shown before color changes after immersion in aqueous khat extract only, and aqueous khat extract with mineral water revealed the highest values, so the mean value of these media was calculated for both tested materials and was further analyzed statistically with T-test, (P < 0.003), 17.6 ± 3.0, 14.3 ± 2.9 for Microhybrid Composite and Nano-Composite respectively (Table 4).

Groups	Difference	
C-group Sub-groups	Mean difference	p-value
HPBS MW	-0.669	1.000
CC	-0.650	1.000
K	-13.37	0.001*
KMW	-13.27	0.001*
KCC	-8.96	0.001*
NPBS MW	-0.949	1.000
CC	-0.305	1.000
K	-10.39	0.001*
KMW	-10.51	0.001*
KCC	-5.09	0.001*

Table 3: *Statistical analysis of the different sub-groups (staining medias) versus the control group (PBS).*
C. group: Control Group; H: Microhybrid Composite; N: Nano-composite.

Subgroups (staining media)	Groups (materials)	GI Microhybrid composite Mean ± St.d	GII Nano-composite Mean ± St.d	p-value
a) PBS		4.1 ± 0.87	3.8 ± 1.1	1.000
b) Mineral Water		4.7 ± 0.47	4.8 ± 1.0	1.000
c) Coca Cola		4.7 ± 0.88	4.18 ± 0.57	1.000
d) Khat		17.8 ± 4.0	14.2 ± 3.3	0.001*
e) Khat with Mineral Water		17.3 ± 1.8	14.3 ± 2.7	0.001*
f) Khat with Cola		13.0 ± 2.2	8.96 ± 1.8	0.001*
Significance among different media		0.001*	0.001*	

Table 4: *Mean ± St. d of color change values (ΔE) of the tested composites after immersion in the different staining media.*

Microhybrid recorded higher value (17.6) than Nano-composite (14.32) with a statistically significant difference of P < 0.003 between both, revealing that, the tested microhybrid composite color was significantly changed than the Nano-composite utilized in this research (Figure 1).

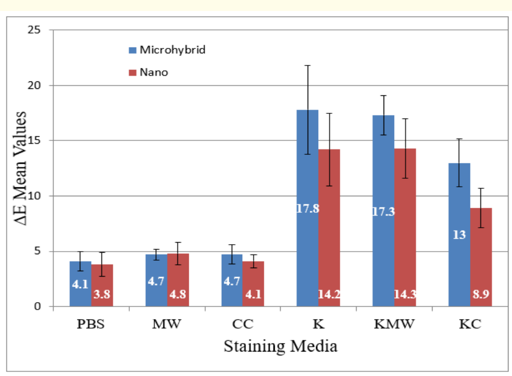

Figure 1: *Mean ΔE value for Microhybrid and Nano-composites in each staining media.*

Discussion

The present study was found a significant difference regarding the color stability between nanofill and microhybrid composite resin, and among different staining media used in this study. These results were in the line with that of previous studies [2,3,16,17]. According to color changes in composite resin materials, three intervals were used to distinguish the values of color changes. These intervals are ΔE < 1 not perceptible by human eyes, ΔE ≤ 3.3 is perceptible by skilled persons, which is acceptable from the clinical point of view and ΔE > 3.3 is easily precepted and observed, so it is not clinically acceptable [18]. Previous studies reported that color changes of composite resin materials in staining solutions showed degree of color changes after 24 hours and 7 days [19], while another studies reported that most obvious discoloration resulted after 14 - 30 days [20], therefore, the pointed out that 14 days could be used as a reference for color comparison. In this study a period of 180 hours was used as a reference for color change comparison for tested materials, with different staining solution. The importance of color stability of esthetic restorative materials is critical for dentists and patients. Therefore, the quality of restoration is considered from both functional and esthetic point of view.

In vitro studies had been done to evaluate the color stability of many resins based and other esthetic restorative materials [21-23]. Type of the staining material and immersion time, were found to be the most significant factors that affect color stability. The variability in the effects of different factors used in the present study was agreed, with the other studies which showed that type of restorative material, polishing system technique and staining media had significant influence on color stability [20]. This work was concerned with the study of the effect of the following staining media (K, MW, CC, KMW and KCC),on color stability of two types of composite materials nanofill (Composan Bio-esthetic) and microhybrid (Composal LCM). Khat chewing habit is common in Yemen, and some eastern African contourites. But up-till my knowledge no published studies concerning color stability has been done. A2 shade of the studied materials was used to minimize the effect of shade variation. To simulate the clinical discoloration, the samples were stored in at 37°C in an incubator [24]. The study samples were polished using Sof-Lex discs, which is believed to provide the smoothest surface of the composite restorations [25]. The time periods selected in this study, is considered as the cumulative time for 180 hours of years of consuming khat, within short period of time, which is suitable for composite resin restoration that can be stained after few years of placement in the oral cavity. Discoloration of composite restorations can be evaluated visually or using instrumental techniques [25]. In the literature most studies used the CIE L* a* b* system to evaluate color stability of composite restorative materials [2,16]. In this study the CIE L* a* b* system was used to evaluate color changes because it is effective in determining small color differences.

In the current study, khat extract was used as a colorant agent in association with other staining solutions, because of its frequent consumption in daily life in Yemen, and some African contourites. The results of the current study revealed that, immersion of microhybrid resin composite samples in different staining media for 180 hours lead to color change of L* value, that was perceptible for K, KMW and KCC indicating discoloration of the study samples, while color changes were not perceptible for PBS, and MW. The mild increase in the L* value after immersion in CC solution, could be related to increased acidity of CC. The response to coca cola among the studied materials, was due to low PH of cola (approximately 2), which could be contributing factor to changes in the color characteristics of the resin materials, cola contain phosphoric acid which is erosive, and hence, it is not clear cola was less aggressive in color changes [26]. Resin based restorations was found to get micro-damage, after storage in acidic media than storage in distilled water or artificial saliva [27]. Similar findings were seen after storage of nanofilled composite restorations in K, KMW and KCC solutions. Lower PH, showed negatively affect wear resistance of composite restorations [28]. However, we did not examine the effect of PH, in color changes in this study. The positive values of a* after storage of microhybrid composites in BPS, K, KMW and KCC staining medias indicates shifting of color towards the red scale. At the same time positive values of a*, was seen in Nano-composites for all staining media, except MW and CC, that showed negative values indicating that, tested composites may have become more translucent, and shifted towards green shade. On the other hand, positive values of b* for both tested composite materials after storage in all staining media indicates color shift towards yellow, therefore, staining may depends on the type of storage solution and its composition [29]. The mean values of color changes (ΔE) for both tested composites after storage in all staining media displayed clinically perceptible (greater than 3.3) color changes, which is in accordance with the results of others [30]. In the present study, the highest values of (ΔE) were seen after immersion of tested materials in K staining media (17.8 for microhybrid), and (14.2 for nanofilled), which was about 5 times greater than the clinically perceptible color. This indicating that, the aqueous khat extract is a powerful staining medium. While the lowest values recorded for PBS (4.1, and 3.8), for microhybrid and nanofill composites respectively, which was expected because PBS represents the artificial saliva, and the small variations in color may be due to temperature, causing increased water uptake by composite, and leaching out of few soluble components of the materials. Khat chewers never spit out the juice, which is extracted during chewing process. Instead they swallow it, and the leaves are left in the oral cavity. Since, khat contains carotene, iron and tin which are the chemical sources of chromospheres (colored agents), so the tendency for khat to cause staining of restoration, among the chronic users is no doubt. The mean values of color change (ΔE) revealed that, there was remarkable change for both tested materials. Comparison between the two tested materials among K, and KMW staining media displayed significant difference denoting that, nanofilled composite material has better color stability than microhybrid composite resin filling material, where there was statistically significant difference, regarding color change values between microhybrid and nanofilled composite filling material, and the difference was visually perceptible, and clinically un-acceptable. High staining values of Composan LCM may be attributed to the high proportion of silane, present in the structure of the material. These results are in agreement with study evaluating the stain ability of reinforced microfill, and microhybrid resin composite restorative materials upon exposure to different staining agents, with the highest color change in microhybrid [30]. On the other hand, it was reported that, the Nano-composite revealed a color stability more than the microhybrid composite, after storage in coffee or red wine solutions [31]. The Nano-composite (composan bio-esthetic) used in this study, contains monomer like Bis-GMA and small portion of hydrophilic monomer TEGDMA. The color change may be to the nature of its matrix. The low TEGDMA content may limit water uptake, and consequently, color variations may be induced by the absorption of the staining media [32]. Likewise, the filler content, size and distribution seems to play an important role in composite color stability and directly correlated to optical properties, and that nanofill particles provide low visual opacity in Nano pigmented dental composites, while filler size might contribute to decrease staining, and enhance esthetic appearance [33]. From this study, the amount of matrix resin could not directly associated with degree of color shift (filler load by volume for Composan LCM is 60% and 56% for Composan bio-esthetic). This is in accordance with the observations, of Dietschi., *et al*. [33] who stated that, a small difference in filler-resin ratio could not be explained by variations in water absorption. In spite of, the short staining period of the present study (180 hours), K and KMW stained seriously the tested materials that had visual perception, consequently, the chewing habit of the patient must be considered while choosing restorative resin-based materials, especially on the anterior region of the mouth. The positive result of the study will help the operator to consider choosing Nano-composite resin, as an option for aesthetic composite resin restoration material. Where it could be considered color stable and resistant to external staining.

It is worth to mention that, it is an arbitrary act to compare the laboratory findings with that of the real intra-oral environment clinical behavior of any restoration, since a number of factors are at play in oral environment, further *in vivo* clinical assessment is suggested.

Conclusion

The authors of the present *in vitro* study concluded that the sustainability of the color of the aesthetic restorations can be gained by considering all mentioned factors and restrict khat chewing habit.

Conflicts of Interest

There are no any conflicts of interest.

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Authors' Contribution

The manuscript was carried out, written, and approved in collaboration with all authors.

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