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RESEARCH ARTICLE

EFFECT OF *CATHA EDULIS* (KHAT) EXTRACT ON COLOR STABILITY OF ACRYLIC RESIN DENTURE TEETH: AN *IN-VITRO* STUDY

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Abstract

Background and aim: Long-term denture tooth color stability for patients is an important criterion in choosing the types of dental materials to use as one of the main concerns in treatment. Discoloration of denture teeth may result in patient dissatisfaction and an additional expense for their replacement. In Yemen, khat (*Catha Edulis*) chewing habit may affect the color of acrylic denture teeth. The aim of this study was to assess the effect of khat extract on color stability of reinforced acrylic resin denture teeth.

Materials and Methods: Experimental design carried to 3 brands of reinforced acrylic resin teeth from three manufacturers, Vitapan, FX-Anterior and Shanghai-SND were evaluated 40 specimens (maxillary central incisors) of each brand were selected systematically and grouped into A, B and C respectively. All samples were stored in distilled water (DW) at 37°C for 24 hours after which baseline colorimetric measurements were recorded by colorimeter. All the specimens were immersed for 24 hours daily and kept in an incubator at 37°C for 30 days. The KH was changed every 24 hours. Colorimeter was used for color change testing of each specimen after 10, 20 and 30 days on CIE L* a* b*. Then the collected data was analyzed using SPSS version 23.

Results: The results of this study shows that there was a significant effect of KH on acrylic teeth (p<0.001). Also, a significant interaction between the three brands acrylic teeth and KH was found (p<0.05). The effect of time on acrylic teeth color stability was statistically significant (p<0.05). The brands teeth specimens at 10, 20 and 30 days in KH revealed more significantly color change than DW (p<0.05).

Conclusions: The khat homogenate (KH) causes discoloration on the 3 brands of reinforced acrylic teeth and the discoloration increases with the passage of time. The change of discoloration is acceptable clinically except in SND teeth. Vitapin teeth has more color stable compared to FX teeth and SND teeth.

Keywords: acrylic denture teeth, color stability, discoloration of denture teeth, khat (*Catha Edulis*), Yemen.

INTRODUCTION

Color is very important in the aesthetic characterization and personalization of a smile. Cosmetic treatment has been one of the most requested dental treatments which come right after pain treatments. Therefore, color stability is an important marker for the clinical success of Prosthodontics. The etiologies of staining of artificial teeth are multifactorial wear, lack of patient care, influence of textural characteristics, exposure to stains, and time are factors that contribute to internal and external staining[1]. Also, in removable dentures, the dentures are an important part of the overall aesthetic outcome. In maintaining aesthetic effects, the color stability of dental prosthesis materials is one of the key factors. Therefore, color stability is an important factor

affecting the longevity of dentures[2]. Several brands of artificial teeth are commercially available and differ in materials such as vacuum sintered porcelain, conventional acrylic resin, and modified resin. Acrylic resin dentures are widely used in oral rehabilitation because they have advantages over porcelain, such as greater resistance to fracture, better absorption of chewing forces, higher flexural impact strength, easier occlusal adjustment, and they offer higher bond strength to the denture base acrylic resin[3]. However, they present low resistance to abrasion and increased susceptibility to color change and biofilm formation[1], which can lead to occlusion unbalance and aesthetic problems and thus have a shorter durability period and service life[1].

Khat plant is an evergreen shrub and cultivated as small tree or a bush. The shrub can grow to reach 6 meters of height approximately with leathery leaves and glossy with brownish green color[4]. Khat (also known as Qat, kat, and Miraa) is the common name for an evergreen plant (*Catha Edulis*), that belongs to the Celastraceae family, endemic to southwest of Arabia and eastern Africa. Millions of Yemenis and East Africans as well as immigrants to the western countries habitually chew the fresh khat leaves and twigs. Khat has many different compounds (alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, minerals and vitamins) the major alkaloids found in fresh plant khat are Phenylalkylamines and cathedulins and they are structurally related to amphetamine[5]. Khat chewing produces stimulating amphetamine-like effects[5]. Based on available information on the association of khat with several reported health and socioeconomic problems affecting consumers and their families in a number of African, Asian, European and North American countries, the WHO Expert Committee on Standards for Pharmaceutical Preparations has recommended the following: khat should be considered as a controlled drug[6]. There is increasing evidence of the negative effects of khat on oral and dental diseases including: white lesions, mucosal pigmentation, periodontal disease, tooth loss, stomatitis, and xerostomia[7-13].

The frequent chewing of khat as a daily habit is the most common habit by Yemeni population, therefore; there is a need to evaluate the effect of chewing khat on the color stability of acrylic resin teeth for long-term clinical success of removable Prosthodontics.

Table 1: The study sample of denture teeth materials.

Type of Materials	Trade Name	Code	Manufacturer	Size	Color	No. of Samples
Reinforced acrylic	Vitapan	V	Vita Zahnfabrik-Germany	large	A1	40
	FX – Anterior	FX	Yamahachi Dental – Japan			40
	Shanghai –SND	SND	Shanghai New Dental- China			40

MATERIALS AND METHODS

Study Design: The study design was an *in-vitro* observational (experimental) study, which carried out in the Laboratory of General Police Hospital, Sana'a City- Yemen. In this study, 120 samples were used to assess and measure the effect of homogeneous khat extract mixture (KH) on color changes of acrylic resin denture teeth.

Simulation Period of the Study: This study was of 30 days (24 h per day or 720 h) as immersion period of Specimens (teeth) in KH would correspond to 6 months continuously of clinical exposure of khat chewing process. Thus, the mean time is 4 hours minimum per day of the Yemeni people who often chew khat from 4-10h per day. Thus, 10 days (240 h) of storage in KH correspond to 2 months of their daily consumption. A 20 days (480 h) of storage correspond to 4 months and 30 days (720 h) of storage correspond to 6 months of consumption.

Sample Size: The sample size was one hundred and twenty (120) acrylic teeth selected and divided into three groups, n=(3×40=120).

Materials: Maxillary (right and left) central incisor denture teeth in Vita shade A1 were selected from three different manufacturers for reinforced acrylic resin denture teeth: Vitapan, FX-Anterior and Shanghai-SND. Maxillary central incisors were used as ready-made of reinforced acrylic molds (block) for each teeth brand used as specimens in this study.

Also, because the maxillary central incisor has a near flat facial surface and has a similar size and shape (uniform surface). The flat face (middle labial) surface of a maxillary central incisor tooth was chosen to measure the degree of discoloration and to ensure that color was properly measured; it was taken from the same spot in the tooth each time as suggested by Gregorius *et al.*,[1]. using a colorimeter (TECHKON-SpectroDens). The size of the specimen (tooth) was large to ensure a more flat surface. Square tooth moulds were selected. The specimens used were selected from shade A1 Vita shade, to exhibit the staining effect more distinctly as suggested by Bayindir *et al.*,[14]. All teeth specimens were examined visually and carefully selected to be free of any defect manufacturer or storing such as porosity and visible fissures, surface irregularities, surface roughness and swelling interface caused by water uptake. The specimens have a very smooth surface with a shiny appearance, especially on the testing side (facial). All the teeth specimens have intact surfaces according to the manufacturer's specifications. All the specimens were stored in a controlled dry environment until the execution of the experiment.

Grouping of the Test Specimens: Three brands of reinforced acrylic resin denture teeth (single shade A1) from three manufacturers were evaluated (grouped as A, B and C respectively). The forty specimens (40 maxillary central incisors) of each brand were selected systematically and randomly divided into two equal

subgroups (20 specimens). The first subgroup was stored in distilled water (DW) as the control group, and the second subgroup was stored in khat homogenate (KH) as the staining media or the study group. So, group A (Vitapan-Vita) was divided into A1 and A2, group B (FX-Anterior Yamahachi) was divided into B1 and B2, and group C (Shanghai-SND New Dental) was divided into C1 and C2. The alphabets indicate the main group and numbers indicate the subgroup. Each specimen was coded with a number by a waterproof pen on the non-testing surface (palatal). So, specimens of each subgroup were coded 1 to 20 to avoid any confusion and ensure color measurement for each specimen. The same sample continues with the same code number during periods of the color measurement on 10, 20, and 30 days as shown in Table 2.

Table 2: Codes of the main group, subgroups and specimens of the acrylic teeth.

Group	Material	Subgroup	Staining media	Experimental codes	Specimens coded
A	V	A1	DW	A1	1 to 20
		A2	KH	A2	1 to 20
B	FX	B1	DW	B1	1 to 20
		B2	KH	B2	1 to 20
C	SND	C1	DW	C1	1 to 20
		C2	KH	C2	1 to 20

Experimental Procedures of the Acrylic Teeth Specimens: All 120 specimens were rinsed with distilled water for 1 minute to remove any debris before immersion. Then each group of teeth specimens was stored (immersed) in distilled water (water bath) at 37°C for 24 hours, in three plastic containers. The initial color measurement (T0) after 24 hours in distilled water, baseline color measurements were taken for each specimen before immersion in KH, using a colorimeter. Before pouring the staining media into containers, six plastic containers, coded with a waterproof pen, were used to store 120 specimens of the teeth. Each container was used to store 20 of the specimens. Each incisor tooth sample was fixed vertically on the floor of the container in the cervical region of each tooth and remains facing tooth exposed to avoid tooth to tooth contact. A carding wax was used to fix the specimen to be later removed easily.

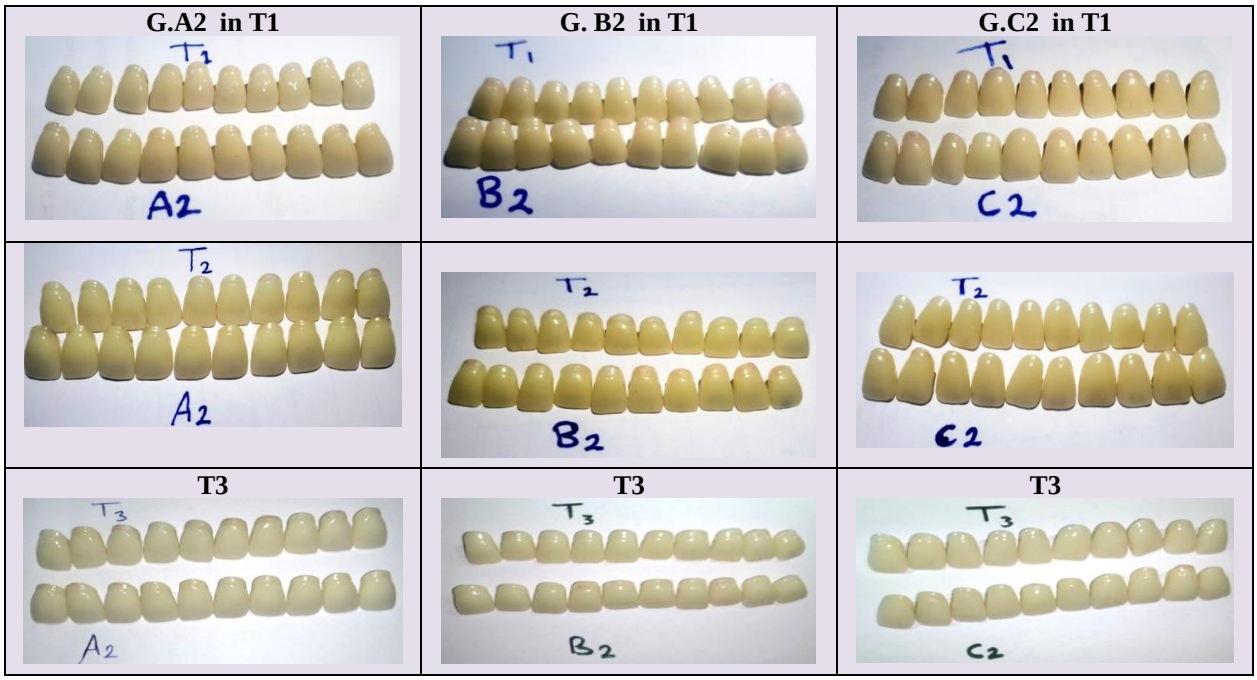

Figure 1: Teeth Specimens (Vitapan=G. A2; FX-Anterior=G.B2 and Shangha-SND.=G.C2) After 10, 20 and 30 Days Immersion in khat Homogenate.

After immersion of the specimens in the respective containers containing the storage staining media. Study group specimens was immersed in the KH and control group specimens was immersed in DW, then all containers were closed and kept in the incubator at 37°C and stored in dark place to simulate oral conditions. The specimens were immersed in KH and DW 24 h a day for 30 days at 37°C as suggested in several *in-vitro* studies[1-6]. To prevent air entrapment around the specimens and reduce the (settling) precipitation of particles in solutions, solutions were shaken every 6 hours for one minute. KH was freshened once every day in each of the three study subgroups during testing period to ensure the activity of crude khat extract and to mimic the process of daily chewing *in vivo*. Overall, KH and DW in all containers were replaced and freshened once after 24 h daily to prevent sedimentation. In addition, the specimens were

washed with distilled water and dried with absorbent paper before the new immersion. KH has the same concentration (1 g/1 ml) and the same amount (33.33 g) in the subgroup containers (A2, B2, C2) until the end of the trial period of 30 days.

Table 3: National Bureau of Standards System of expressing color difference.

NBS unit	Critical remarks of color differences	
0.0 – 0.5	Trace	Extremely slight change
0.5 – 1.5	Slight	Slight change
1.5 – 3.0	Noticeable	Perceivable change
3.0 – 6.0	Appreciable	Marked change
6.0 – 12.0	Much	Extremely marked change
12.0 or more	Very much	Change to other color

To investigate color change of specimens after immersion, the color measurements were repeated after 10 days (T1), 20 days (T2) and 30 days (T3) as suggested by Singh and Agarwal[15] as the Figure 2. After each session of color measurements that was recorded in Excel spreadsheet.

Preparation of Khat Extract: Fresh Hamdani khat was used for homogeneous khat extract mixture preparation (KH). The fresh leaves and twigs of khat were cleaned with running water, air-dried, and packed in plastic bags and transported to the Lab. Homogeneous khat extract mixture (KH) was prepared by adding 100 grams of fresh minced khat which has been extracted with 100 ml distilled water (W/V) at a ratio of 1:1. The khat homogenate (KH) was then mixed with 2gm of NaOH until; its pH becomes similar to the pH of saliva and the oral cavity, as suggested by Al Moaleem *et al.*,[6]. Afterward, it was shaken at 160 rpm, in the shaking machine for 10 minutes at room temperature, which helps to speed up mixing. Khat homogenate (KH) was daily prepared and distributed and equally divided into 3 containers (A2, B2, C2) and the same concentration (1g/mL) in the containers for the 30 days. Samples were washed with distilled water and dried with absorbent paper before fresh immersion.

Colorimeter Readings: Color measurement was conducted before and after immersion. Before immersing each group of denture teeth into the KH, they were stored in distilled water at 37°C for 24 hours. After 24 hours of immersion, the color of each tooth (T0) was measured in daylight with a colorimeter (TECHKON SpectroDens). The color of each tooth was measured in daylight with a colorimeter on a black background in order to simulate the absence of light in the mouth as suggested by Colombo *et al.*,[16]. Color measurements were repeated after 10 days (T1), 20 days (T2) and 30 days (T3) of immersion in either the staining media (KH) or in the control solution (DW). Before each measurement session, the colorimeter was calibrated according to the manufacturer's recommendation by using the supplied white calibration standard A. Each specimen was measured twice, and then the average value was calculated and recorded. This value was considered the average color ΔE*. Color changes were characterized using the Commission International d'Eclairage L*a*b* color space (CIE L*a*b*).

Calculation of Color Changes

The color change in the different CIE L* a* b* axes (ΔL*, Δa*, Δb*).The total color change (ΔE*) was calculated for each specimen at a specific time interval of 10,20, and 30 days, relative to its baseline color using the color difference formula: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ where ΔE* represents the color difference and ΔL*, Δa*, Δb* represent the changes in lightness, changes in the red-green coordinate, and changes in the yellow-blue coordinate respectively. Therefore, total color differences were expressed by the formula: $E^* = [(L)^2 + (a)^2 + (b)^2]^{1/2}$, where L, a and b is different in L*, a* and b* values before (T0) and after immersion at each time interval (T1, T2 and T3). After each session of color measurements, the data stored in an excel spreadsheet. To link the amount of color change (ΔE*) recorded by the colorimeter to a clinical environment, the data (ΔE*) were converted to national bureau of standards units (NBS units) using the equation: NBS unit = ΔE*×0.92, where important remarks of color differences are represented in NBS units (Table 3).

Data Analysis: All data were entered into an excel spreadsheet using Excel 13 computer software. Total color changes (ΔE*) is the difference between the two colors (baseline and after immersion at 10, 20 and 30 days. All the intervals were calculated using a calculator for calculate Delta-E (Cie76 algorithm). *http://colormine.org/delta-e-calculator*. Mann-Whitny test was used to compare the quantitative data with abnormal distribution at 10 day or 20 days or 30 days on the two materials. Kurskal-Wallis test was used to compare the quantitative data with abnormal distribution at 10 days or 20 days or 30 days on the three materials. Frinedman test was used to compare the quantitative data with abnormal distribution at 10 days or 20 days or 30 days on the three materials. One-way ANOVA test was used to compare the quantitative data with normal distribution at three times on three materials.

RESULTS

The National Bureau of Standards System (NBS) units are calculated to assess the color differences caused by immersion time and/or solution type. A total color change expressed by an ΔE* value is converted to the NBS unit using the equation, NBS unit = ΔE*×0.92, to link the color change with clinical environment in terms of NBS units, as shown in Table 3. So, there is slight change in all of ΔE 10 days for three brands (Vitapan acrylic teeth, FX-Anterior acrylic teeth and Shanghai-SND acrylic teeth) after immersion in KH (mean ΔE 10 days of 0.9, 1.2 and 1.5 respectively) which is clinically acceptable, and trace change (extremely slight change) in all of ΔE 10 days for three brands (groups) after immersion in distilled water (mean ΔE 10 days of 0.3, 0.4 and 0.5 respectively), which is clinically acceptable. Table 4 shows that

there are differences in the mean and standard deviation of the total color changes (ΔE*) for the Vitapan acrylic teeth, FX-Anterior acrylic teeth and Shanghai-SND acrylic teeth over 10 days, 20 days and 30 days after immersion in khat homogenate (KH) as a study group and distilled water (DW) as a control group. The second immersion period, days 20, shows a gradual increase in the total color change relative to days 10 (Noticeable change) for all three companies (Vitapan acrylic teeth, FX-Anterior acrylic teeth and Shanghai-SND acrylic teeth) after immersion in KH (mean ΔE 20 days of 1.9, 2.2 and 2.5 respectively) which is clinically acceptable, and slight change in of ΔE 20 days for two companies after immersion in distilled water except trace change in Vitapan acrylic teeth (mean ΔE 20 days of 0.6, 0.7 and 0.9 respectively), which is clinically acceptable (Table 4).

Table 4: Color Differences of the acrylic teeth after 10, 20 and 30 days.

Type of Teeth	Group	Sub-G (n=20)	Staining media	Time	Mean ΔE*	S.D	NBS unit
Vitapan	**A**	A1 Control	DW	ΔE10days	0.3	0.05	(0.2) Trace
				ΔE20days	0.6	0.09	(0.5) Trace
				ΔE30days	0.9	0.05	(0.8) slight change
		A2 Study	KH	ΔE10days	0.9	0.11	(0.8) slight change
				ΔE20days	1.9	0.18	(1.7) Noticeable
				ΔE30days	3.1	0.41	(2.8) Noticeable
FX-Anterior	**B**	B1 Control	DW	ΔE10days	0.4	0.04	(0.3) Trace
				ΔE20days	0.7	0.12	(0.6) slight change
				ΔE30days	1.1	0.13	(1.0) slight change
		B2 Study	KH	ΔE10days	1.2	0.26	(1.1) slight change
				ΔE20days	2.2	0.31	(2.0) Noticeable
				ΔE30days	3.3	0.68	(3.0) Appreciable
Shanghai-SND	**C**	C1 Control	DW	ΔE10days	0.5	0.05	(0.5) Trace
				ΔE20days	0.9	0.07	(0.8) slight change
				ΔE30days	1.3	0.13	(1.1) Slight change
		C2 Study	KH	ΔE10days	1.5	0.36	(1.3) Slight change
				ΔE20days	2.5	0.66	(2.3) Noticeable
				ΔE30days	3.7	0.86	(3.4) Appreciable

DW=Distilled Water ; KH=Khat Homogenate; ΔE10, ΔE20 and ΔE30=the magnitude of color difference of the samples after 10 days, 20 days, and 30 days, respectively; ΔE*=Total color change value; SD=Standard Deviation ; NBS unit=National Bureau of Standards unit= ΔE* × 0.92 ; Sub G=Subgroup; n=number of simples.

The third immersion period, days 30, shows appreciable color changes (marked change) for two companies (FX-Anterior acrylic teeth and Shanghai-SND acrylic teeth) after immersion in KH except noticeable change in Vitapan acrylic teeth (mean ΔE 30 days of 3.1, 3.3 and 3.7 respectively), which is clinically acceptable except Shanghai-SND acrylic teeth more than 3.3 which is clinically unacceptable, and showed slight color changes in of ΔE 30 days for three companies after immersion in distilled water (mean ΔE 30 days of 0.9, 1.1 and 1.3 respectively), which is clinically acceptable (Table 4). The color change is smaller in the 10 days but increased by prolongation of immersion time. The greatest color change occurs at 30 days and was the highest in shanghai-SND acrylic teeth followed by FX-Anterior acrylic teeth and Vitapan acrylic teeth in KH (mean ΔE of 3.1, 3.3 and 3.7 with a standard deviation of 0.41, 0.68 and 0.86 respectively), as shown in Table 4 and Figure 2. Table 5 shows a comparison of the mean change in ΔE values within the teeth study group and control group when immersed in KH and DW at 10, 20 and 30 days. There is statistically significance difference in color within Vitapan, FX-Anterior and Shanghai-SND to different immersion periods at 10, 20 and 30 days either control group or study group with p =0.001. The color change is at 10, 20 and 30 days either in control group or in the study group increased by prolongation of immersion time but more increased in the study group (KH). The greatest color change occurs at 30 days within one group of denture teeth either in the control group or in the study group and the highest is in Shanghai-SND teeth followed by FX-Anterior teeth and Vitapan teeth in KH (median ΔE 30 days of 3.4, 3.1 and 2.9 respectively) and in DW (mean ΔE 30 days of 1.3, 1.1 and 0.9 respectively) (Table 5).

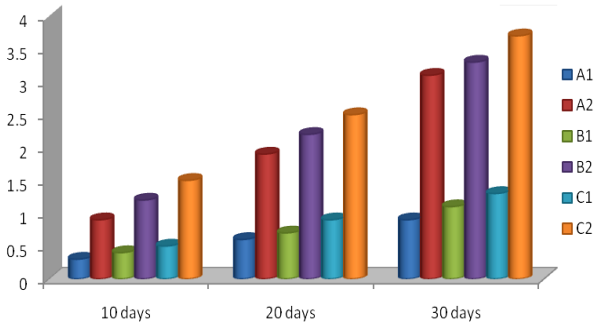

Figure 2: Mean Color Changes (ΔE* values) of Acrylic Teeth after 10, 20 and 30 days of Immersion in khat Homogenate as a Study Group and the Distilled Water as a Control Group.

The statistical difference in color is explained by Post Hoc analysis between 10 days and 20 or 10 and 30 days within the three groups. The color change is at 10, 20 and 30 days either in the control group or in the study group, increased by prolongation of immersion

time, but more increased in the study group (KH) (Table 5). Bonferroni test statistical analysis (Table 5) indicates mean difference of the total color change in acrylic denture teeth is statistically significant differences (p<0.05) affected by either KH or DW. The different immersion periods at (10 days to 20 days) and (10 days to 30 days) and (20 days to 30 days) affect the color stability of acrylic teeth as revealed by a pair-wise comparison. Table 6 shows the comparison of mean change in ΔE values between the three brands (3 groups). There is statistically significant difference between Vitapan, FX-Anterior and Shanghai-SND at ΔE 10, 20 and 30 days either control group or study group where p value was 0.001. The statistical difference in color is explained by Post Hoc analysis between Shanghai-SND and Vitapan, Shanghai-SND and FX-Anterior, statistical difference in color change between Vitapan, FX-Anterior and Shanghai-SND at ΔE 10, 20 and 30 days either control group or study group where p value is 0.001.

Table 5: Comparison within one group of acrylic teeth to different immersion periods.

Type of Teeth	Time	N	Control Groups (Distilled Water)		Study Groups (Khat Homogenate)	
			Mean ± SD	*p*	Median (Rang)	*p*
Vitapan	ΔE10 days	20	0.3 ± 0.04	0.001*	0.95 (0.83 - 1.25)	0.001**
	ΔE20 days	20	0.6 ± 0.09		1.9 (1.7 - 2.4)	
	ΔE30 days	20	0.9 ± 0.05		2.9 (2.7 - 3.9)	
FX-Anterior	ΔE10 days	20	0.4 ± 0.04	0.001*	1.1 (0.9 - 1.8)	0.001**
	ΔE20 days	20	0.7 ± 0.1		2.1 (1.9 - 3.2)	
	ΔE30 days	20	1.1 ± 0.1		3.1 (2.3 - 4.6)	
Shanghai-SND	ΔE10 days	20	0.5 ± 0.05	0.001*	1.3 (1.1 - 2.5)	0.001**
	ΔE20 days	20	0.9 ± 0.07		2.2 (1.8 - 4.1)	
	ΔE30 days	20	1.3 ± 0.1		3.4 (2.7 - 5.1)	

Multiple comparisons by Bonferroni (Post Hoc) test.

Type of Teeth	Time	Control Groups (Distilled Water)		Study Groups (Khat Homogenate)	
		Mean Difference	*p*	Mean Difference	*p*
Vitapan	10-20	-0.31	0.001	-0.95	0.001
	10-30	-0.59	0.001	-2.09	0.001
	20-30	-0.28	0.001	-1.14	0.001
FX-Anterior	10-20	-0.33	0.001	-1.08	0.001
	10-30	-0.67	0.001	-2.17	0.001
	20-30	-0.34	0.001	-1.09	0.001
Shanghai-SND	10-20	-0.39	0.001	-1.02	0.001
	10-30	-0.76	0.001	-2.25	0.001
	20-30	-0.37	0.001	-1.22	0.001

Time=or days; p=Probability value≤0.5=Significance

Bonferroni test statistical analysis (Table 7) indicates mean difference of the color change between the three brands denture teeth (3 groups) with the same immersion period at ΔE 10, 20 and 30 days is p<0.05 significantly affected by either KH or DW and denture teeth manufacturer, excepted between FX-Anterior and Shanghai-SND at ΔE 20 days and 30 days is not significant (p>0.05) (mean difference of 0.3 and 0.2 respectively) affected by either KH and denture teeth manufacturer. The type of denture teeth materials affects the color stability at each immersion period and in each staining media, as revealed by a pair-wise comparison) (Table 7). Table 8 shows comparison of mean color change in values between study group and control group in the same group at the same 10, 20 and 30 days when immersed in distilled water (DW) and Khat Homogenate (KH). There are statistically significant differences between the mean of E* for control group and study group in all groups where p value is <0.05 level.

Table 6: Comparison between the three groups in the same immersion period.

Groups	Time	N	Control Groups (Distilled Water)		Study Groups (Khat Homogenate)	
			Mean ± SD	*p*	Median (Rang)	*p*
A-B-C	ΔE10 days	60	0.3±0.04 0.4±0.04 0.5±0.05	0.001*	0.9(0.8- 1.3) 1.1 (0.9 – 1.8) 1.3 (1.1 – 2.5)	0.001**
A-B-C	ΔE20 days	60	0.6±0.09 0.7±0.1 0.9±0.07	0.001*	1.9 (1.7 – 2.4) 2.1 (1.9 – 3.2) 2.2 (1.8– 4.1)	0.001**
A-B-C	ΔE30 days	60	0.9±0.05 1.1±0.1 1.3±0.1	0.001*	2.9 (2.7 – 3.9) 3.1 (2.3 – 4.6) 3.4 (2.7 – 5.1)	0.02**

A=Teeth (Vitapan); B=Teeth (FX-Anterior); C=Teeth (Shanghai-SND); N=number of samples; *=One Way Anova test; **=Kurskal Wallis test; p=Probability value p≤0.5=Significance.

Khat Homogenate (KH) causes the largest changes in ΔE than distilled water for Vitapan acrylic teeth, FX-Anterior acrylic teeth and Shanghai-SND acrylic teeth at ΔE 10, 20 and 30 days.

DISCUSSION

The values of the three brands of reinforced acrylic teeth tested (Vitapin, FX-Anterior and Shanghai-SND), when immersed in KH for 30 days (720h) were (a mean ΔE value of 3.1, 3.3 and 3.7 respectively), meaning that the color change in the Vitapin teeth was a noticeable (perceivable change) whereas the color change in FX-Anterior and Shanghai-SND was an appreciable (marked change). Overall, the behavior of all tested teeth immersed in KH for 30 days could be considered clinically unacceptable and in disagreement with NBS and the ΔE* of 3.0 is clinically acceptable. In addition, this study coincides with the values (ΔE* 3.1 and 3.3) obtained for Vitapin teeth and FX-Anterior teeth respectively would be clinically acceptable, except for the values of Shanghai-SND teeth (ΔE* 3.7) which could be considered clinically unacceptable compared to other studies[17,18] which concluded that ΔE* values of ≥1 to ≤3.3 can be detected by the eyes and are clinically acceptable.

Table 7: Multiple comparisons by Bonferroni (Post Hoc) test.

Groups	Time	Control groups (Distilled Water)		Study Groups (Khat Homogenate)	
		Mean Difference	*p*	Mean Difference	*p*
A-B	ΔE10 days	-.10	0.001	-2.6	0.009
A-C		-.19	0.001	-5.2	0.001
B-C		-.09	0.001	-3.7	0.001
A-B	ΔE20 days	-.13	0.001	-3.8	0.001
A-C		-.28	0.001	-3.6	0.001
B-C		-.19	0.001	-1.03	0.3
A-B	ΔE30 Days	-.19	0.001	-1.7	0.09
A-C		-.37	0.001	-2.7	0.008
B-C		-.18	0.001	-1.4	0.2

A=Teeth (Vitapan); B=Teeth (FX-Anterior); C=Teeth (Shanghai-SND); *p*=Probability value $p \leq 0.05$=Significance

Table 8: Comparison between the control and the study for the same group at the same immersion period.

T	N	Group A			Group B			Group C		
		S.G	Median (Rang)	*p*	S.G	Median (Rang)	Sig.	S.G	Median (Rang)	*p*
10	40	A2	0.95(0.8-1.2)	0.001*	B2	1.1 (0.9-1.8)	0.001*	C2	1.3(1.1-2.5)	0.001*
		A1	0.3(0.2-0.4)		B1	0.4 (0.3-0.5)		C1	0.5(0.4-0.6)	
20	40	A2	1.9(1.7-2.4)	0.001*	B2	2.1(1.9-3.2)	0.001*	C2	2.2(1.8-4.1)	0.001*
		A1	0.6(0.4-0.8)		B1	0.7(0.5-0.9)		C1	0.9(0.8-1.1)	
30	40	A2	2.9(2.7-3.2)	0.001*	B2	3.1 (2.3-4.6)	0.001*	C2	3.4(2.7-5.1)	0.001*
		A1	0.9(0.8-1.0)		B1	1.0 (0.9-1.3)		C1	1.3(1.0-1.5)	

T=Time or days; N=Number of sample; S.G=Subgroups; A2=Study Group (khat); A1= Control Group (distal water); *p*=Probability value ≤ 0.5=Significance; *=Mann-whitney test

The effect of KH on the color of tested teeth was evident in all teeth to different degrees. The ΔE* values were slightly higher, as shown in Table 4, with mean ΔE values of 30 days of 3.1, 3.3 and 3.7 for Vitapin, FX-Anterior and Shanghai-SND, respectively. This result agrees with the finding of Al-Anesi *et al.*,[19] Who stated that color change due to exposure to khat solution is very superficial and can be easily removed by teeth brushing and occurs due to deep absorption of stains and thus cleaning is more difficult. Also, there was a possibility that the mix of KH with NaOH might have generated an acidic pH solution, weakening the surface integrity of the teeth and creating good conditions for discoloration. Since, khat contains carotene, iron and tin, which are the chemical sources of chromospheres (colored agents); the tendency for that khat to cause staining restoration, among the chronic users is no doubt[19]. On the other hand, crude khat has tannin and some amount of fluoride, which may be the cause of staining[4].

The current study revealed in Table 5 there was a statistically significant difference in the effect of KH on Vitapin teeth and FX-Anterior teeth and Shanghai-SND teeth in different immersion periods of 10, 20 and 30 days. The result explained that the increase in color changes with time where $p \leq 0.05$. This result was accompanied with a study that was carried out by Mousavi *et al.*,[20] who mentioned that there was statistically significance about the effect of tea, cola and coffee on the reinforced acrylic teeth which, $p \leq 0.05$ in three weeks and six weeks but disagrees with periods of one week, six weeks for tea where $p > 0.05$.

The current study in Table 6 explained that there was a statistically significant difference in color changes that was explained by Post Hoc analysis between the three brands in the effect of KH on Vitapin teeth and FX-Anterior teeth and Shanghai-SND teeth in the same immersion period. When comparison was made among the denture tooth materials, the Vitapin teeth have less color changes than FX-Anterior teeth and Shanghai-SND teeth in KH and can be presented in the following

decreasing order: Vitapin teeth (most stable)>FX-Anterior teeth>Shanghai-SND teeth at 10, 20 and 30 days were statistically significance difference between the three groups $p \leq 0.05$. This result was in agreement with the study carried out by Koksal and Dikbas,[21] who mentioned that there was a statistically significant difference of $p \leq 0.05$ between the three brands of reinforced acrylic teeth after one day and one month of immersion in tea, coffee and coke solution. It could be due to different absorption properties of materials, different polarity, and different hydrophilic nature and different manufactures of the brands acrylic teeth. The present study showed in Table 8 that pair-wise comparisons of the average total color change between two solutions of homogenization of khat (KH) as a study group and distilled water (DW) as a control group for the same acrylic denture teeth tested in each over a period of 10, 20, and 30 days, Vitapin teeth, FX anterior teeth, and Shanghai-SND teeth had significant differences ($p<0.05$) affecting color stability in solution type. The reaction of the solution factor type is significant in each time period. This result was also similar to the study by Koksal and Dikbas[21].

The current study explains the color change in distilled water as a control group with three brands of acrylic teeth's mean was ΔE 30 days of 0.9, 1.1 and 1.3 for Vitapin, FX-Anterior and Shanghai-SND, respectively, having imperceptible least color changes with values of ΔE* less than 1.3. Generally, in this study, after 30 days distilled water as a control demonstrated the least effect on the color stability and small color change occurred in all teeth tested. This result was in agreement with those obtained by Moon *et al.*,[2] and Gregorius *et al.*,[1] may be due to the thermal effect of the immersion temperature related to intrinsic factors which include loss of fillers and matrix, which might happen following a period of storage in water or saliva and/or due to acrylic polymers absorb water[22].

Limitation of the study

This study was based on evidence-based research method. Barriers to applying evidence-based dentistry in an accurate manner include time, insufficient training, and lack of relevance to practice as in current study was laboratory-only. Obtaining sufficient evidence of the harms of khat to dentures in practice can be improved by producing a method based on evidence-based clinical practice.

CONCLUSIONS

The khat homogenate (KH) causes discoloration on the 3 brands of reinforced acrylic teeth and the discoloration increases with the passage of time. The change of discoloration is acceptable clinically except in SND teeth. Vitapin teeth has more color stable compared to FX teeth and SND teeth. There is an effect of KH at 10, 20 and 30 days within the same brand and between brands for V, FX and SND.

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AUTHOR'S CONTRIBUTIONS

This research is part of a master's degree in the Prosthodontics Department, Faculty of Dentistry, Sana'a University, Yemen. **Mohammed BSA:** writing original draft, lab work. **Al-Kebsi AM:** methodology, investigation. **AL-Haddad KA:** data analysis, report drafting. **Al-Sanabani NF:** methodology, data curation. **Al-Najhi MMA:** review, and editing, methodology. **Al-hamzi AHY:** conceptualization, methodology. **Al-Shamahy HA:** editing, supervision. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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