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Assessment of Color Stability of Bleached Enamel Surface After The Application Of Some Natural Antioxidants For Different Times

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ABSTRACT

Background: Residual free radicals following bleaching may disrupt adhesion processes, necessitating the application of antioxidants to neutralize these radicals. Nonetheless, concerns persist about the potential for antioxidants to change the color of bleached enamel. **Objective:** To assess the effect of two natural antioxidants, Moringa oleifera and Salvadora persica, applied at varying time intervals (10, 15, and 20 minutes) on the color of bleached enamel.

Methods: Seventy-two non-carious human premolars extracted for orthodontic reasons were chosen. The crowns were sectioned, embedded in acrylic resin, and polished to provide a consistent enamel surface. All samples were subjected to bleaching with 40% hydrogen peroxide gel and subsequently categorized into six experimental groups according to the type of antioxidant (Moringa oleifera or Salvadora persica) and application duration (10, 15, or 20 minutes). Color measurements were conducted prior to and after to the application of antioxidants utilizing a digital colorimeter based in the CIELAB system.

Results: Statistical analysis indicated no significant color changes (ΔE) in any of the groups post-antioxidant administration, with all ΔE values remaining beneath the clinically visible threshold. The antioxidant type and treatment duration did not have a statistically significant effect on enamel color ($p>0.05$).

Conclusion: The application of Moringa oleifera and Salvadora persica as post-bleaching antioxidants does not affect the color of bleached enamel. These results validate that natural antioxidants can be utilized post-bleaching without compromising cosmetic results.

Keywords: *Dental whitening, antioxidants, Moringa oleifera, Salvadora persica, enamel chromatic stability, CIELAB system.*

1. INTRODUCTION

As awareness of aesthetic dentistry increases, bleaching has emerged as a prominent process among the several treatment options available to meet this desire as a conservative, safe, effective, and non-invasive treatment [1]. It is important to note that hydrogen peroxide (HP) is the active ingredient in most bleaching agents. This powerful oxidizing agent breaks the substantial, pigmented molecules responsible for tooth discoloration into smaller, less pigmented entities [2]. Nonetheless, the adverse effects of bleaching on tooth pulp, periodontal tissues, and enamel morphology have been documented [3]. Post-bleaching, the enamel surface exhibits persistent oxygen free radicals that impede the photopolymerization of resin dental materials, leading to a reduction in enamel bond strength [4]. The detrimental effect can be mitigated within a period of 1 to 3 weeks [5], such a delay could have significant outcomes in a clinical setting. Unexpected results, like tooth fracture, may result from postponing the restorative process [6].

Numerous techniques have been suggested to mitigate the oxygen free radicals from enamel bleached surface, including the removal of the superficial enamel layer, pretreatment of bleached enamel with alcohol, utilization of adhesives containing organic solvents, implementation of a post-bleaching waiting period of 24 hours to three weeks, and the application of antioxidants. Numerous studies have demonstrated that both natural and synthetic antioxidants are a safe and effective means to enhance bond strength immediately following bleaching [7]. In recent years, natural antioxidants have garnered interest for their capacity to neutralize residual free radicals, therefore enhancing post-bleaching color stability. Compounds like

green tea polyphenols, grape seed extract, and vitamin C exhibit notable antioxidant capabilities that may mitigate post-bleaching discolouration. The efficacy of these natural antioxidants may fluctuate based on their application timing and duration [8].

Moringa Oleifera functions as a natural antioxidant. The phenolic components of Moringa Oleifera leaves directly influence its free radical scavenging activity [9]. Methanol and ethanol were identified as the most effective solvents for extracting antioxidant chemicals from Moringa leaves [10]. Salvadora persica (Siwak) has numerous biological activities, including notable antibacterial, antifungal, antioxidant, and anticariogenic effects, mostly via elevating plaque pH following a sugar exposure [11]. It is chosen as an example of natural remineralizing compounds [12] that were found to be more effective in remineralizing enamel lesions than sodium fluoride (NaF) in a prior study. They possess varying concentrations of calcium and phosphate, in addition to fluoride [13]. Tooth color is defined by color coordinates according to the universal color specification and standardized system developed by the CIE (Commission Internationale de l'Eclairage, or the International Commission on Illumination), which are reasonably easy to read. The primary variables are lightness (L*), red (+a*), and yellow (+b*) [14].

The Commission International de l'Éclairage (CIE) Lab system (CIELAB) is a uniform color space (UCS) authorized by CIE in 1976 and later released as a Joint ISO/CIE Standard [15]. It has transformed color assessment in dental bleaching by providing an objective framework for evaluating and quantifying changes in tooth color. Founded in 1976, the technology was developed beyond previous efforts to correlate visual perception with quantifiable data [16]. This study aims to assess the impact of different natural antioxidants (Salvadora Persica and Moringa Oleifera) on the color stability of bleached enamel at three different application time intervals (10 min., 15 min., 20 min.), providing insight into their potential role in maintaining long-term aesthetic outcomes after tooth whitening procedures.

2. MATERIAL AND METHODS

The ethical approval was received from the "Research Ethical Committee" at the Faculty of Dentistry, Mosul University, Iraq, at clearance number (RCE reference no. UoM.Dent. 24/1008.

Preparation of Specimens: Seventy-Two healthy premolars (extracted for orthodontic purposes from patients aged 16 to 20 years) were examined to ensure they were devoid of any cracks, caries, or other enamel defects, were cleaned with an ultrasonic scaler (Rundeer, China) and disinfected using a 0.1% thymol solution. The teeth crowns were transversely sectioned (2 mm) beneath the cementoenamel junction using a two-sided diamond carbide disc (Zhengzhou smile, China) with a continuous water supply to ensure efficient cooling throughout the process, and each tooth crown was then affixed in acrylic resin with the labial surface oriented upwards [17]. The central area of the labial surface was leveled with 600-grit silicon disc (Zhengzhou smile, China) with a standard position handpiece affixed to a locally modified adjusted horizontally graduated microscope base to provide optimal standardization (figure 1), so producing a smooth, flat enamel surface for all tooth samples (figure 2). The flat enamel surface of each specimen had been examined under a stereomicroscope (Optika, Ponteranica BG, Italy) at 30x to ensure it was devoid of any flaws, fissures, or exposed dentin [18].

Figure 1. A standard position handpiece affixed to a locally modified adjusted horizontally graduated microscope base.

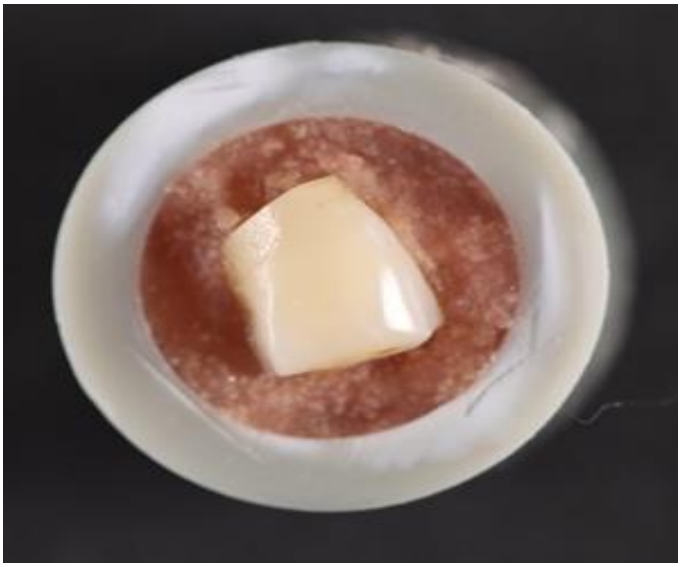

Figure 2. The tooth crown sample with flat labial surface.

Sample grouping: A total of 72 teeth specimens will randomly be allocated according to surface treatment material and application time into six experimental groups (n = 12):

Group 1: Teeth bleaching+ Moringa oleifera extract application for 10 min. (B+MO 10 min.)

Group 2: Teeth bleaching+ Moringa oleifera extract application for 15 min. (B+MO 15 min.)

Group 3: Teeth bleaching+ Moringa oleifera extract application for 20 min. (B+MO 20 min.)

Group 4: Teeth bleaching+ Salvadora persica extract application for 10 min. (B+MO 10 min.)

Group 5: Teeth bleaching+ Salvadora persica extract application for 15 min. (B+MO 15 min.)

Group 6: Teeth bleaching+ Salvadora persica extract application for 20 min. (B+MO 20 min.)

All groups will be tested for color change analysis after 24 hours, all groups tested before and after the application of antioxidants materials.

Antioxidant Preparation:

Preparation of Moringa oleifera alcoholic extract (MOE): Leaves of Moringa oleifera (MO) were harvested from a local farm in Erbil City, Iraq and the roots of S. persica (Salvadoraceae), known as Arak, were imported from Jeddah, Saudi Arabia, both were identified, and extracted in the Department of Biochemistry, College of Education, University of Mosul, Iraq.

The extraction of both plants (root of Salvadora Persica and Moringa Oleifera leaves) was conducted by washing the fresh leaves and roots with distilled water, shade-drying them at room temperature (22-25°C), and grinding them into powder using an electric grinder (Silver Crest, Germany). The powder (200 g) was initially defatted with 2000 ml of petroleum ether (boiling range 60-80) (scharlab S.L., Spain) and agitated for 24 hours using a locally manufactured modified mechanical stirrer. Subsequently, the mixture was filtered through Whatman filter paper size No. 1 (Double rings, China) and allowed to dry at room temperature [19]. The dried powder was immersed in 2000 cc of 70% ethanol and agitated with a mechanical stirrer for 72 hours at room temperature (22-25°C) in complete darkness. The extract will thereafter undergo two filtration processes utilizing Whatman filter paper size No. 1 (Double rings, China). The solvent will be entirely eliminated utilizing a rotary evaporator (GWSI, Zhengzhou, China). The extract was removed and preserved in tubes at 4°C [20,21].

2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) Assay of Antioxidant: To make the standard solution of vitamin C and the sample (antioxidant), 0.5 grams of vitamin C (scharlab S.L., Spain) were combined with 100 milliliters of each methanol and distilled water. The standard solution concentration was 5000 ppm, and by utilizing the dilution law, various concentrations of vitamin C and samples were created at 30, 60, 120, 250, and 500 ppm [22]. The mixture was agitated rapidly using a vortex mixer (Infitek co., China) and permitted to rest at room temperature for 30 minutes in darkness. Subsequently, absorbance was quantified at 517 nm utilizing a UV-VIS spectrophotometer (Shimadzu, china) [23]. The inhibitory concentration (IC 50 value) of the sample (the concentration necessary to inhibit 50% of the DPPH free radical) was determined using a logarithmic dose-inhibition curve. A reduced absorbance of the reaction mixture signified increased free radical activity [24].

The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100.

Where A0 was the absorbance of blank (Vitamin C) and A1 was the absorbance in presence of test sample [25].

Bleaching Procedure: A (1 mm) layer of the Power Whitening YF 40% HP supplied with an auto mixing tip was applied to the enamel surface for 20 minutes over three successive cycles, as per the manufacturer's instructions. Following each cycle, the teeth will be meticulously cleaned with water for 60 seconds and subsequently air-dried for 30 seconds [26]. Following the bleaching technique, samples from all groups will be preserved in artificial saliva for 24 hours prior to testing. During the experimental period, the teeth were stored in artificial saliva at 37°C simulating oral conditions. The artificial saliva was prepared and changed each day, and a digital pH meter (Juanjuan, China) was used to check the pH of the solution [27].

Application of antioxidants and Bonding Procedures: Following the bleaching procedure, the flat enamel labial surface will be designated with light-cured resin barrier [28]. Subsequently, a syringe will be utilized to administer 1 ml of each antioxidant in accordance with their respective group (figure 3) for the assigned duration [29]. The surfaces will subsequently be cleaned with distilled water for 30 seconds and dried with compressed air for 5 seconds [30].

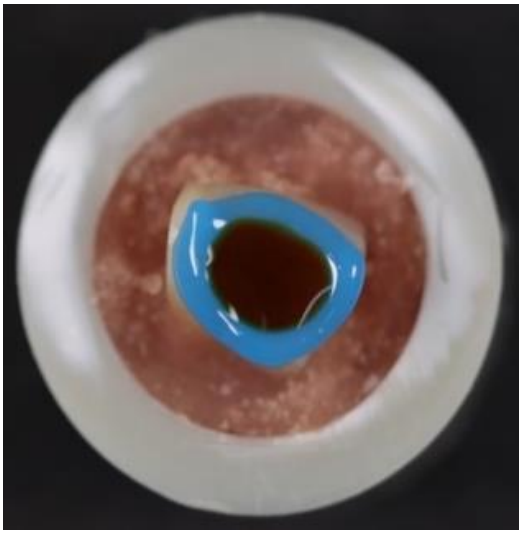

Figure 3. An antioxidant extract solution on labial surface of tooth.

Color stability measurements: Enamel color measurements for all teeth samples will be objectively recorded using a digital hand-held colorimeter (NR110 3nh, China). Color data was determined in accordance with the CIE L*a*b* system. To ensure the fixed position of the colorimeter device tip, the device was vertically affixed to a metal laboratory clamp that oriented the tip downward. The sample diameter's position was delineated on the clamp's floor to ensure all samples were measured from the same location in direct contact with the colorimeter tip that was perpendicular to the buccal surface of the tooth (figure 4). All measurements were conducted by one operator at 9 AM on a white background. A resultant data displayed on colorimeter digital screen using the following equation [31, 32]:

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

Where dL*, da*, and db* shows the variation in L*, a*, and b* values, respectively [33].

•ΔE (mean color difference) >3.7 – easily visible difference.

•ΔE between 3.7 and 1 – clinically acceptable difference.

•ΔE <1 – difference clinically not visible.

Color coordinates (L, a and b) of each tooth were measured at two different stages as follows [34]:

1. Immediately after bleaching procedure.

2. Immediately after antioxidant extracts application.

Figure 4. The colorimeter, sample standardizing, and final result display. a. NR110 colorimeter device that has been fixed to the clamp. b. the standardized sample position. c. The digital screen that displayed the Final results.

3. RESULTS

2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) Assay of Antioxidant:

The antioxidant activity (AA%) of the antioxidant extracts was evaluated using a dose-dependent assay across various concentrations (30 ppm to 500 ppm), as shown in Table 1.

Table 1. Total antioxidant activity (%) in both natural antioxidant extracts compared to vitamin C.

	30 ppm	60 ppm	120 ppm	250 ppm	500 ppm
Vit C	12.00	24.15	41.98	63.81	70.25
Salvadora Persica	22.14	31.58	54.56	70.14	83.12
Maringa Oleifera	25.44	49.58	64.58	79.80	91.25

At all concentrations, the results recorded that Moringa oleifera exhibited superior antioxidant activity than Salvadora persica extract. At the lowest concentration (30 ppm), the antioxidant activity (AA%) was 22.14% for Salvadora persica and 25.44% for Moringa oleifera. At higher concentrations, the activity increased significantly. For instance, at 250 ppm, the antioxidant activity reached 70.14% and 79.80% for Salvadora persica and Moringa oleifera, respectively. At the maximum concentration tested (500 ppm), Moringa oleifera achieved an AA% of 91.25%, markedly higher than Salvadora persica, which reached 83.12%. The antioxidant activity of both extracts surpassed that of the standard reference of antioxidant (Vitamin C), which exhibited an AA% of 70.25% at 500 ppm.

Enamel surface color stability: The data were verified to be normally distributed using Shapiro-wilk test. The normality analysis was revealed that the color change (ΔE) values is normally distributed among antioxidant extract types and different times of antioxidant application, therefor, only parametric statistical analysis tests were used.

Descriptive statistical analysis which includes (minimum, maximum, mean and standard deviation) of color change (ΔE) values of all groups was calculated and represented in (Table 2).

Table 2. The descriptive statistical analysis of color change ΔE for all tested antioxidant groups regarding the times of applications.

Antioxidant extracts	**Application Time (min.)**	**N**	**Min.**	**Max.**	**Mean±SD**
Moringa oleifera	10	12	0.40	0.90	0.7250±0.13568
	15	12	0.50	1.20	0.8083±0.23916
	20	12	0.60	1.30	0.8833±0.18505
Salvadora persica	10	12	0.50	1.20	0.7667±0.21881

	15	12	0.60	1.10	0.8250±0.14848
	20	12	0.80	1.50	1.1250±0.25271

N: sample number, SD: standard deviation.

The highest mean of color change (ΔE) was observed in the Salvadora persica (20 min. application time) group (1.1250 ± 0.25271), and the lowest mean was observed in the Moringa (10 min. application time) group (0.7250 ± 0.13568). Other groups showed proximity in the color change. Generally, the color change coefficient varies directly with the increase in application time. The ΔE for all groups are (<1 – difference clinically not visible) except the Salvadora persica (20 min. application time) group is (ΔE between 3.7 and 1 – clinically acceptable difference).

Two-way ANOVA test for color change among the groups is shown in (Table 3).

Table 3: Two-way analysis of variance (ANOVA) of the test groups.

Source of variance	Type III Sum of Squares	df	Mean Square	F	Sig.
Type of antioxidant	0.180	1	.180	4.433	0.039*
Time of application	0.855	2	.428	10.531	0.000*
Type * Time	0.182	2	.091	2.247	0.114 NS
Error	2.680	66	.041		
Total	56.600	72			
Corrected Total	3.898	71			

NS: non-significant. *: Significant.

It revealed statistically significant difference within the type of antioxidant groups and significant difference among the time of application periods at($p \leq 0.05$). And there is no statistically significant difference at their interaction level on the color change assessment.

Duncan's multiple range test for the effect of the application times and type of antioxidant extract on the enamel color change (Table 4)

Table 4. The effect of application times and type of antioxidants on means of color change (ΔE) for all groups.

Antioxidant application time		Moringa persica	Salvadora persica	General mean
10 min.	(ΔE) mean	0.7250 [b]	0.7667 [b]	0.7458 [b]
	No.	12	12	24
	SD	0.13568	0.21881	0.17932
15 min.	(ΔE) mean	0.8083[b]	0.8250 [b]	0.8167 [b]
	No.	12	12	12
	SD	0.23916	0.14848	0.19486
20 min.	(ΔE) mean	0.8833 [b]	1.1250 [a]	1.0042 [a]
	No.	12	12	12
	SD	0.18505	0.25271	0.24931
Total	(ΔE) mean	0.8056	0.9056	
	No.	36	36	

	SD	0.19704	0.25961	

The result showed no significant differences between all tested groups related to the application times regarding the types of antioxidants except the Salvadora persica extract at (20 min.) application time producing a significantly higher color change. But it within the clinically acceptable difference category. Generally, the moringa persica produce the lower color change values.

4. DISCUSSION

When bleaching agents are applied to dental structures, they generate free radicals such as nascent oxygen and hydroxyl radicals. A highly reactive molecule that has one unpaired electron referred as a free radical. They may result in micro-morphological defects in bleached enamel and reduce resin bonding, therefore, literature focused on the application of antioxidant after bleaching to repair demineralized lesion by absorbing the free oxygen radicals, and as a result, boosting the oxidation/reduction reaction that occurs on the surface of the enamel [35].

Sound human permanent premolar teeth from comparable patient age were selected for this study, due to the ease of their collection, less variation in morphology, the adequate thickness of enamel on the buccal surface, fewer chances of microcracks in young tooth, and avoid age related changes of enamel. The color assessment was done on the middle third of the teeth crowns to exclude the thin enamel layer on the cervical third and the thick one on the occlusal third. Artificial saliva was used as a storage media because it is regarded as a chemical reservoir of calcium and phosphate which mimic the behavior of human saliva [36].

The Moringa Oleifera and Salvadora Persica have been choice in this study as plants that contain natural antioxidants such as flavonoids, phenolics, and carotenoids with beneficial antioxidant, antibacterial, and free radical scavenging capabilities that they possess. [9, 12]

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay (AA%) provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time [37]. The antioxidant activity indicated that both Moringa oleifera and Salvadora persica extracts have considerable free radical scavenging capabilities. The Moringa oleifera demonstrated greater an antioxidant activity in comparison with that of Salvadora persica's and both of them surpassing the standard reference, Vitamin C at the corresponding concentrations.

Recent researches support these conclusions that indicated the ethanolic extracts of Moringa oleifera leaves demonstrate significant antioxidant activities related to its abundant elevated levels of bioactive components, particularly phenolics and flavonoids [38]. Another study indicated that methanolic extracts of Salvadora persica fruits have significant antioxidant activity, attributed to their total phenolic and flavonoid contents [39].

The colorimeter is the highest reliable standard for color matching. according to CIELAB system denotes color change which is popular and widely used and was introduced for determination of colors for clinical evaluation. The color difference can be distinguished by the general population when value $\Delta E > 3.7$ and is contemplated to be clinically acceptable and perceivable, and hence it is applied in most of the color-related studies in dentistry [40].

This study evaluated the color stability of bleached enamel after the application of two natural antioxidants, Moringa oleifera and Salvadora persica, at various time intervals (10, 15, and 20 minutes), to ascertain if the color of the antioxidants affected bleached enamel shading. The findings indicated that neither of the used antioxidants induced a substantial color alteration since all ΔE values were below the clinically detectable threshold ($\Delta E < 3.7$), reinforcing the assertion that the inherent color of antioxidants does not influence enamel color stability.

These findings corroborate with previous study which indicated that natural antioxidants did not affect the color stability of bleached enamel, so reinforcing the theory that the use of antioxidants post-bleaching does not result in apparent discoloration [41]. This supports earlier research, which revealed that specific antioxidants, including those with pigmented molecules, do not inherently cause enamel discoloration [33]. In addition to Degirmenci et al. (2020) who discovered that several antioxidants affected color stability, and their influence varying based on the formulation and duration of exposure, which aligns with the minor rise in (ΔE)noted for Salvadora persica during extended application times [34].

A reasonable explanation for the slight elevation in (ΔE) with extended application durations is that prolonged exposure may augment enamel permeability, thereby modifying light interaction with the surface; however, our findings demonstrate that even after 20 minutes, the color alteration remained within clinically acceptable parameters. A crucial factor affecting color stability is the washing protocol after antioxidant application; a 30-second rinse effectively eliminated loosely bound pigments and residues, preventing their adherence to enamel, a method corroborated by prior research highlighting the significance of adequate post-application rinsing in preventing pigmentation [42, 43]. The utilization of alcohol in antioxidant extraction resulted in little discoloration, since alcohol enhances pigment breakdown and evaporates rapidly, hence reducing pigment contact with enamel and promoting adhesion to the enamel surface [44, 45].

While this study provides strong evidence that the color of natural antioxidants does not affect bleached enamel, several limitations should be noted. First, this study was conducted in vitro, which may not fully replicate the dynamic oral environment, including salivary flow, dietary habits, and mechanical forces like chewing and brushing. Second, only short-term color stability was assessed, and long-term studies are necessary to determine whether these antioxidants maintain their protective effect over weeks or months. Third, pH levels of the antioxidants were not measured, which could influence enamel permeability and surface changes. Fourth, only two antioxidants (Moringa oleifera and Salvadora persica) were evaluated, whereas future research should compare multiple antioxidants with different chemical compositions.

5. CONCLUSION

In conclusion, this study confirms that both natural antioxidant extracts (Moringa Oleifera and Salvadora Persica) have potent antioxidant activity and their using does not compromise the color stability of bleached enamel, as their impact on discoloration remains clinically insignificant. Their minimal interaction with enamel, along with effective cleansing mechanisms, prevents noticeable staining, making them a viable post-bleaching treatment. Additionally, their biocompatibility and potential to neutralize residual peroxide radicals further support their integration into modern aesthetic dentistry. These findings contribute to the growing body of evidence advocating for natural antioxidants as a safe and effective approach to maintaining enamel integrity and aesthetic outcomes after bleaching procedures.

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