Original Article / Özgün Araştırma

The Antioxidant Effect of Green Tea, Rosemary, and Their Combination on Resin Bond Strength to Bleach Tooth Structures

Ağartılmış Diş Dokularına Rezin Bağlanma Dayanımında Yeşil Çay, Biberiye ve Bunların Kombinasyonunun Antioksidan Etkileri

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Abstract

Objective: This study aimed to evaluate the effect of four experimental antioxidant protocols on the shear bond strength of a resin-based composite to bleach the enamel and dentin.

Materials and Methods: Using extracted bovine incisors, 140 enamel/140 dentin specimens were prepared. Both enamel and dentin samples were assigned into seven groups, individually (n=20): ENC/DNC= negative control, EPC/DPC= positive control, EDR/DDR= delayed restoration, ESA/DSA= sodium ascorbate, EGT/DGT= green tea, ER/DR= rosemary and EGTR/DGTR= green tea and rosemary combination. Experimental antioxidant solutions prepared from sodium ascorbate, green tea, or rosemary extracts were applied to the bleached enamel/dentin samples in the ESA/DSA, EGT/DGT and ER/DR groups, respectively. The mixture of the green tea/rosemary extract solutions at a 1:1 ratio was applied to the EGTR/DGTR groups to investigate possible synergistic antioxidant interaction. The shear bond strength (SBS) test was conducted at a crosshead speed of 0.5 mm/minute. Failure modes were assessed under a stereomicroscope at x40 magnification. Data were analysed statistically using Welch-ANOVA and Tamhane post-hoc tests.

Results: The lowest and highest mean SBS values were obtained in the positive control groups (EPC/DPC) and negative control groups (ENC/DNC), respectively ($p<0.05$). Delaying of the composite resin restorations for 15 days improved bonding to the bleached enamel/dentin compared to the positive control groups ($p<0.05$). All the antioxidant protocols, except green tea and rosemary combination, exhibited a bonding strength that was statistically similar to that of the relevant delayed restoration groups ($p>0.05$). Synergistic antioxidant interaction could not be obtained in the green tea and rosemary combination protocol.

Conclusion: Natural plant-derived antioxidants can be an alternative to synthetic sodium ascorbate and may enable immediate resin restorations of bleached tooth structures.

Öz

Amaç: Bu çalışmanın amacı, ağartılmış mine ve dentine kompozit rezin bağlanma dayanımında dört farklı antioksidan tedavi protokolünün etkisini değerlendirmektir.

Gereç ve Yöntemler: Çalışmada 280 adet çekilmiş sığır keser dişinden 140'ar adet mine ve dentin örneği elde edildi. Mine ve dentin örnekleri kendi aralarında yedişer gruba ayrıldı (n=20): ENC/DNC= negatif kontrol, EPC/DPC= pozitif kontrol, EDR/DDR= ertelenmiş restorasyon, ESA/DSA= sodyum askorbat, EGT/DGT= yeşil çay, ER/DR= biberiye, EGTR/DGTR= yeşil çay ve biberiye kombinasyonu. Sodyum askorbat, yeşil çay ekstraktı, biberiye ekstraktı ve yeşil çay ve biberiye ekstrakt kombinasyonundan %10 konsantrasyonda hazırlanan deneysel antioksidan solüsyonları ESA/DSA, EGT/DGT, ER/DR ve EGTR/DGTR gruplarında yer alan örneklerin ağartılmış mine/dentin yüzeylerine uygulandı. Makaslama bağlanma dayanımı (MBD) analizi, kafa hızı 0,5 mm/dakika olarak ayarlanan test cihazında gerçekleştirildi. Kırılma tipleri, stereomikroskop altında x40 büyütme ile değerlendirildi. Verilerin istatistiksel analizinde Welch ANOVA ve post-hoc Tamhane testleri kullanıldı.
Bulgular: En düşük ve en yüksek MBD değerleri pozitif kontrol (EPC/DPC) ve negatif kontrol (ENC/DNC) gruplarında bulundu ($p<0,05$). Kompozit rezin restorasyonların 15 gün süreyle ertelenmesi (EDR/DDR) ağartılmış mine/dentine bağlanmayı pozitif kontrol gruplarına kıyasla arttırdı ($p<0,05$). Yeşil çay ve Biberiye kombinasyonu hariç tüm antioksidan protokollerinde, ertelenmiş restorasyon gruplarına benzer düzeyde bağlanma dayanımı elde edildi ($p>0,05$). Yeşil çay ve biberiye kombinasyon protokolünde sinerjistik antioksidan etki elde edilemedi.
Sonuç: Bitkisel kaynaklı doğal antioksidanlar, sentetik sodyum askorbata alternatif olabilir ve ağartılmış diş dokularının rezin içerikli materyallerle hemen restore edilebilmesini sağlayabilirler.

Introduction

Tooth bleaching is a conservative, rapid, and low-cost option for the esthetic treatment of discolored teeth. In case when the bleaching treatment could not fulfill the esthetic expectations of the individuals, additional restorative treatment may be needed, or existing restorations may have to be renewed (1). Nevertheless, extensive research has shown that the bond strength between freshly bleached tooth structures and the resin-based restorative materials is compromised (2-8). This problem was primarily due to the decomposition by-products of HP, which are residual oxygen and free radicals released in the form of hydroxyl, per-hydroxyl, and superoxide anions (9,10). For these by-products to be removed from the oxidized tooth structures, a waiting period of 1-3 weeks was recommended in the literature (1). From a clinical perspective, delaying the restorations for a certain time and increasing the number of sessions may not be well-tolerated by the individuals expecting for their treatment to be concluded in a shorter time (11,12).

Immediate application of the antioxidants to freshly bleached tooth surfaces has been recommended to benefit from their free radical scavenging activities (1). Antioxidants are compounds that can neutralize free radicals by donating their electrons. Upon application of the antioxidants, residual oxygen and the free radicals trapped within oxidized tooth structures are removed and consequently, the compromised bonding of the resin-based restorative materials to tooth structures is improved. Immediate post-bleaching application of the antioxidants can facilitate the resin-based adhesive restorative procedures to be completed in the same session and reduce the need for another dental visit (1,13).

Among various antioxidants, 10% sodium ascorbate has been the most widely investigated antioxidant in laboratory studies (13). Sodium ascorbate is the sodium salt of ascorbic acid, also known as vitamin C. Although its free radical scavenging/antioxidant activity on oxidized enamel/dentin has been demonstrated in numerous *in vitro* studies (1-3,12-14), sodium ascorbate application remains an experimental approach that has no place in the clinical setting. The disadvantage of using sodium ascorbate is that it is highly sensitive to ambient conditions such as heat, light, oxygen, humidity, pH and that it loses its stability and effectiveness rapidly (15).

Recently, researchers have focused on natural plant extracts to develop a non-toxic, biocompatible, and effective antioxidant protocol which can be applied safely to oxidized dental structures. Flavonoids, phenolic compounds or their derivatives in the composition of the natural plant extracts can prevent auto-oxidation via different mechanisms such as reducing or inhibiting oxygen formation, free radical scavenging, or metal ion chelating (16). Several natural antioxidant sources (1,4-6,17) have been investigated, yet there lacks a plant-derived natural antioxidant protocol developed for use in daily clinical practice.

As the natural plant-derived antioxidants, freshly prepared green tea (Camellia sinensis) and rosemary

(Rosmarinus officinalis L.) extracts were investigated in this study, in terms of their free radical scavenging effects on bleached enamel/dentin.

Despite their well-documented antioxidant effects in literature, research related to the free radical scavenging effects of the green tea and rosemary extracts on the oxidized tooth structures is quite limited (4,5,17).

Research in the pharmaceutical and food industry have demonstrated that synergistic interaction resulting from the combination of antioxidant plant extracts or isolated pure compounds can be beneficial. The mixture of phytochemical and/or synthetic bioactive compounds may exert greater bioactivity than a single compound and become more effective in the oxidation process (16,18-20). Previously, no study has investigated the possible synergistic antioxidant interaction on the oxidized tooth structures. In this study, to investigate any possible synergistic interaction on bleached enamel/dentin, experimental antioxidant solutions prepared from green tea and rosemary extracts were combined in a 1:1 ratio. To the best of our knowledge, this is the first study that investigates synergistic antioxidant interaction that could be advantageous in neutralizing free radicals within oxidized tooth structures.

This study aimed to comparatively evaluate three different natural antioxidant protocols prepared from green tea extract, rosemary extract, and their 1:1 combination as well as a synthetic antioxidant protocol prepared from sodium ascorbate (SA) in terms of their effects on improving compromised bond strength to bleached enamel/dentin. Those hypotheses were tested in this study:

1. All experimental antioxidant protocols applied after bleaching will improve enamel-resin or dentin-resin shear bond strength, compared to positive control.

2. There will be no difference between the antioxidant protocols in terms of improving bond strength to bleached enamel/dentin.

Materials and Methods

Preparation of the Enamel and Dentin Samples

G-Power v.3.1.9.2 software (Heinrich Heine, University of Düsseldorf, Düsseldorf, Germany) was used to determine the required minimum sample size according to the data of a previous research (21). Based on the parameters of an alpha-type error of 0.05, a beta power of 0.95, and an effect size of 0.40, the minimal estimated sample size per group was found to be 20. Considering the 7 individual groups for both enamel and dentin samples, 280 teeth in total (140 enamel samples and 140 dentin samples) were included in the study.

Two-hundred-eighty bovine incisors obtained from a slaughterhouse were used in the study. Extracted teeth were cleaned from soft tissue remnants with a periodontal curette under running water and then

Table 1. Enamel groups created according to the treatment protocols

Enamel groups (n=20)	Bleaching	Antioxidant protocol	Restoration delay time
Enamel-negative control	-	-	-
Enamel-positive control	40% HP 20 min x3	-	-
Enamel-delayed restoration	40% HP 20 min x3	-	15 days
Enamel-sodium ascorbate	40% HP 20 min x3	10% Sodium ascorbate, 10 min	-
Enamel-green tea	40% HP 20 min x3	10% Green tea, 10 min	-
Enamel-rosemary	40% HP 20 min x3	10% Rosemary, 10 min	-
Enamel-green tea and rosemary	40% HP 20 min x3	10% Green tea & rosemary, 10 min	-

HP: Hydrogen peroxide, min: Minute

disinfected by immersing in the 0.1% thymol solution for 1 week. The teeth were kept in distilled water until the experiment and used within one month.

Each tooth was separated from its cementoenamel junction by using a diamond separator equipped with a handpiece and a micromotor working at the slow speed. The roots were removed away, and the crowns were divided into two segments as facial and lingual, by cutting at the inciso-apical direction. The facial tooth segments were embedded in auto-polymerizing acrylic resin placed in polyvinyl chloride molds with enamel surfaces facing upward.

Of all the tooth segments, 140 were used directly as enamel samples. The remaining 140 teeth were converted to dentin samples by removing their enamel layer with a #010 diamond bur equipped with an aerator under water cooling. The enamel/dentin surface of each sample was wet-grinded with a 600-grit silicon carbide paper for 15 s to obtain a standard smear layer and a flat surface. Enamel and dentin samples were divided individually into 7 groups, according to the treatment protocols (Tables 1, 2). Each group consisted of 20 enamel/dentin samples (n=20):

ENC/DNC: Enamel/dentin negative control groups. No bleaching or antioxidant was applied.

EPC/DPC: Enamel/dentin positive control groups. Restorations were performed immediately after bleaching treatment. No antioxidant was applied.

EDR/DDR: Enamel/dentin delayed restoration groups. After bleaching, all samples were kept in distilled water in an incubator, at 37 °C for 2 weeks. No antioxidant was applied.

ESA/DSA: Enamel/dentin sodium ascorbate groups. After bleaching, all samples were immediately treated with the sodium ascorbate antioxidant solution.

EGT/DGT: Enamel/dentin green tea groups. After bleaching, all samples were immediately treated with the green tea antioxidant solution.

ER/DR: Enamel/dentin rosemary groups. After bleaching, all samples were immediately treated with the rosemary antioxidant solution.

EGTR/DGTR: Enamel/dentin green tea and rosemary combination groups. After bleaching, all samples were immediately treated with the green tea and rosemary combination solution.

Bleaching Treatment of the Enamel and Dentin Samples

Except for the samples in negative control groups, ENC and DNC, all samples were treated with in-office bleaching (Opalescence Boost PF, Ultradent, Inc., South Jordan UT, USA) according to the manufacturer's instructions. Forty percent HP containing bleaching gel was applied on the enamel/dentin surfaces at 1-mm-thickness. The bleaching gel was left in place undisturbed for 20 minutes on the enamel samples or 10 minutes on the dentin samples. Afterward, the

Table 2. Dentin groups created according to the treatment protocols

Dentin groups (n=20)	Bleaching	Antioxidant protocol	Restoration delay time
Dentin-negative control	-	-	-
Dentin-positive control	40% HP 10 min x3	-	-
Dentin-delayed restoration	40% HP 10 min x3	-	15 days
Dentin-sodium ascorbate	40% HP 10 min x3	10% Sodium ascorbate, 10 min	-
Dentin-green tea	40% HP 10 min x3	10% Green tea, 10 min	-
Dentin-rosemary	40% HP 10 min x3	10% Rosemary, 10 min	-
Dentin-green tea and rosemary	40% HP 10 min x3	10% Green tea and rosemary, 10 min	-

HP: Hydrogen peroxide, min: Minute

bleaching gel was removed gently with a gauze patch and the bleached surfaces were rinsed with distilled water for 60 s. The bleaching procedure was repeated in 3 consecutive applications. Bleached enamel and dentin samples in delayed restoration groups (EDR/DDR) were kept in the incubator at 37 °C within distilled water for 15 days.

Preparation of the Green Tea and Rosemary Extracts

Pure plant extracts (Camellia sinensis and Rosmarinus officinalis L.) were used in the preparation of experimental antioxidant solutions in this study. Taxonomical identification of the plants was performed in the Department of Biology, Faculty of Arts and Sciences, Aydın Adnan Menderes University.

Twenty-five grams of dried green tea sample and 200 mL of ethanol were placed into a beaker. The beaker was sealed with the parafilm and shaken on a bench-top shaker (Promax 2020, Heidolph, Kelheim, Germany) at 120 rpm for 2 h. Afterward, all ingredient within the beaker was transferred into a blender and mixed. The resulting mixture was filtered through a black ribbon filter paper and the (first) filtrate was kept in the dark at +4 °C. Subsequently, the green tea residue was treated with 200 mL of ethanol and shaken under the same conditions for 24 h. The (second) filtrate and the residue were separated by filtration. Repeatedly, 100 mL of ethanol was added on the plant residue, shaken for another 2 h and filtrated as described above. The residue was discarded, and the filtrates obtained at the end of three successive filtration processes were combined. Thus, the phenolic compounds within the composition of green tea were extracted into ethanol. The ethanol was removed from the filtrate in a rotary evaporator (RE, IKA RV 05 basic 1B, Staufen, Germany) at +40 °C. The extract solution was transferred from the evaporator flask to the petri dish, by dissolving in approximately 10.0 mL of ethanol, and then dried in the incubator at +40 °C for 12 h, to remove all solvent. Freshly prepared green tea extracts were kept at -18 °C, until use.

Thirty-nine grams of the rosemary sample and 300 mL of ethanol were placed into a beaker. The beaker was sealed with the parafilm, and then subsequent procedures were repeated as described for the preparation of green tea extract.

Preparation and the Application of the Experimental Antioxidant Solutions

The amounts of the plant extracts and sodium ascorbate powder were weighed on an analytical balance (Radwag, AS 220/C/2, Radom, Poland). Distilled water was measured using an automatic pipette (Isolab Pipette by CAPP, Wertheim, Germany).

Experimental 10% sodium ascorbate solution was prepared by dissolving 10 g of sodium ascorbate powder (Sigma-Aldrich, St. Louis, USA) in 100 mL distilled water. Its pH value was measured with a pH meter (Hanna, pH211, Rhode Island, USA) (pH=7.76).

Ten grams of green tea extract and 100 mL distilled water were combined in a glass beaker. The beaker was sealed with the parafilm and the mixture was stirred on a heated magnetic stirrer (Promax 2020, GmbH & Co KG, Kelheim, Germany) at 70 °C for 15 min. Then the glass beaker was taken into the ultrasonic bath (Apple, Ultrasonic LC 30, Germany) and incubated for 15 min under ultrasonic vibration. Following this step, the mixture was transferred to microcentrifuge tubes and blended for another 15 min in a centrifuge (Sigma 3-30 K, Germany). At the end of the spinning period, the mixture was passed through filter paper twice to remove the precipitate and the filtrated liquid was used as the 10% green tea antioxidant solution in the study (pH=5.69).

To prepare the 10% rosemary antioxidant solution, 10 gr rosemary extract and 100 mL of distilled water were taken into a glass beaker. All other procedures were performed as described above for the preparation of green tea antioxidant solution. (pH=5.23).

Ten percent green tea and 10% rosemary solutions were mixed in a ratio of 1:1 and 10% green tea & rosemary combination solution was prepared (pH=5.33).

All experimental antioxidant solutions were kept in tightly closed glass jars in the refrigerator at +4 °C until the experiment and used within a week. Antioxidant solutions were applied to the bleached enamel/dentin surfaces of the samples in the experimental antioxidant groups ESA/DSA, EGT/DGT, ER/DR, and EGTR/DGTR. Application of the antioxidant solutions was performed actively by rubbing on the enamel/dentin surfaces with a microbrush for 10 min, and the solutions were refreshed each min. Then the samples were rinsed with distilled water for 20 s.

Composite Resin Restoration

The enamel and dentin surfaces of the samples were conditioned with 35% phosphoric acid gel (K-Etchant, Kuraray, New York, USA) for 30 s or 15 s, respectively, and then rinsed with distilled water thoroughly. Enamel samples were dried using an air-water syringe. A gentle airstream was applied to dentin samples for 4-5 s, to keep dentin surfaces humid. Two-step etch & rinse adhesive system (Adper Single Bond 2, 3M ESPE, St. Paul, MN, USA) was applied in two consecutive layers on sample surfaces according to the manufacturer's instructions. Each adhesive layer was rubbed onto the sample surface with a microbrush for about 15 s. Next, the adhesive applied surfaces were gently air-streamed for 5 s to remove the solvent and then polymerized for 10 s with an LED light-curing unit (Monitex, New Taipei City, Taiwan).

Transparent plastic tubes (4-mm-height x4-mm-width) employed as the templates were positioned at the center of each sample surface. A 2-mm-thick micro-hybrid composite resin layer (Filtek Z250, 3M ESPE, St. Paul, MN, USA) was inserted within each template and then light-cured for 20 s. The second composite resin layer was applied and light-cured for 20 s. The templates were removed with a scalpel, and composite resin restorations were light-cured for an additional 20 s. Following the completion of the build-up restorations, all samples were transferred into distilled water and kept in an incubator (Hera Therm, Thermo Fisher Scientific Co., USA) at 37 °C for 24 h. The contents of the materials used in the study are given in Table 3.

Shear Bond Strength Test

Shear bond strength was measured with a shear test device equipped with a software (Mode Dental, Esetron Smart Robotechnologies, Ankara, Turkey). First, the data regarding the restoration surface area (12.56 mm^2), the maximum load (500N), and the crosshead speed (0.5 mm/min) were entered into the software and then, each sample was connected to the test device. SBS values at the moment of fracture were recorded automatically as N and MPa (N/mm^2) (Tables 4, 5).

The fracture interfaces of the samples were examined at x40 magnification under a stereomicroscope (Olympus SZ61, Munster, Germany) equipped with an imaging system (Olympus cellSens Standard, Munster, Germany). The fracture types were recorded as adhesive, cohesive, or mixed. The adhesive fracture represented the failure that occurred at enamel/adhesive or dentin/adhesive interface. The cohesive fracture represented the failure that occurred within the composite resin, dentin, or enamel. The mixed fracture was defined when the failure occurred both in adhesive and cohesive types. The numbers and the percentages of the fracture types are given in Table 4 and Table 5.

Statistical Analysis

SPSS 25 software (IBM SPSS Statistics, Armonk, NY, USA) was used for the statistical analysis. The SBS data of the enamel and dentin groups were compared within themselves, yet not between each other. Lilliefors corrected Kolmogorov-Smirnov test, orthogonality-skewness coefficients, and histogram were used to determine the suitability of the data

Table 3. Contents of the materials used in the study

Brand	Material	Composition	Manufacturer
Filtek Z250 LOT: N842589	Microhybrid composite resin	Inorganic fillers (%60), Bis-GMA, UDMA, Bis-EMA	3M ESPE, St. Paul, MN, USA
K-Etchant LOT: 2Q0035	Acid gel	35% phosphoric acid	Kuraray Co., Osaka, JAPAN
Adper Single Bond 2 LOT: N853720	Two-step etch&rinse adhesive resin	Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator system, methacrylate functional copolymer of polyacrylic and polyitaconic acids, silica nanofiller	3M ESPE, St. Paul, MN, USA
Opalescence Boost PF LOT: M1080108	In-Office bleaching gel	40% hydrogen peroxide gel, fluoride, potassium nitrate	Ultradent, South Jordan UT, USA
Sodium L-ascorbate LOT: BCBT8088	Synthetic antioxidant	L(+)-Ascorbic acid sodium salt C6H7NaO6, BioXtra, ³ %99	Sigma- Aldrich, St. Louis, USA

to normal distribution. Levene's test was used to analyze the homogeneity of variances. Since the data were distributed normally, yet the variances were not homogeneous, the Welch ANOVA test was performed. Tamhane post-hoc test was used to evaluate the differences between the groups. Significance was evaluated at the level of $p<0.05$.

Results

The mean, standard deviation (± SD) SBS value (in MPa) and fracture types of the enamel and dentin groups are shown in Table 4 and Table 5.

The lowest and highest mean SBS values were obtained in positive control groups (EPC/DPC) ($p<0.05$) and negative control groups (ENC/DNC) ($p<0.05$), respectively. The lowest and highest rate of adhesive type fractures were observed in negative control groups (ENC/DNC) and positive control groups (EPC/DPC), respectively.

The mean SBS value measured in the delayed restoration groups (EDR/DDR) were significantly higher ($p<0.05$), and the rate of the adhesive type failures were lower compared to the positive control groups. The mean SBS data measured in the enamel delayed restoration group (EDR) did not reach the level of enamel negative control group (ENC) ($p<0.05$). The mean SBS data measured in the dentin delayed restoration (DDR) reached to a level that was statistically similar to the dentin negative control group (DNC) ($p>0.05$).

All antioxidant-applied enamel and dentin groups exhibited relatively higher SBS values compared to EPC or DPC, respectively ($p<0.05$). None of the antioxidant-applied enamel groups reached the SBS value that was statistically similar to ENC ($p<0.05$). Of all antioxidant-applied dentin groups, only the sodium ascorbate group presented the SBS value that was statistically similar to DNC ($p>0.05$).

All antioxidant-applied enamel and dentin groups, except green tea and rosemary combination, exhibited the SBS value that was statistically similar to EDR or DDR, respectively ($p>0.05$).

Discussion

In the present study, both the enamel and dentin samples in the positive control groups (EPC/DPC) exhibited lower bond strength data and a higher rate of adhesive type fracture compared to other groups. These findings were consistent with other studies (2-8) revealing the deterioration in resin bonding efficacy to freshly bleached dentin.

Table 4. Mean, standard deviation, minimum, maximum SBS value (in MPa) and fracture types of the enamel groups

Enamel groups (n=20)	SBS value (MPa)	Fracture types		
	Mean (±SD)*	Adhesive n, (%)	Cohesive n, (%)	Mix n, (%)
ENC	21.63 (±1.71)[a]	10 (50%)	4 (20%)	6 (30%)
EPC	7.14 (±1.35)[b]	15 (75%)	0 (0%)	5 (25%)
EDR	18.47 (±2.02)[c]	12 (60%)	2 (10%)	6 (30%)
ESA	18.40 (±2.59)[c]	12 (60%)	2 (10%)	6 (30%)
EGT	16.23 (±2.74)[cd]	14 (70%)	1 (5%)	5 (25%)
ER	15.96 (±3.01)[cd]	14 (70%)	1 (5%)	5 (25%)
EGTR	14.92 (±2.44)[d]	14 (70%)	0 (0%)	6 (30%)

*Significant differences between means are characterized by different lowercase letters ($p<0.05$).

ENC: Enamel negative control groups, EPC: Enamel positive control groups, EDR: Enamel delayed restoration groups, ESA: Enamel sodium ascorbate groups, EGT:Enamel green tea groups, ER: Enamel rosemary groups, EGTR: Enamel green tea & rosemary combination groups, SD: Standard deviation, n: Number

Post-bleaching delay of restorations for 15 days could improve bonding to dentin (DDR) to the level that is statistically similar to the dentin-negative control group (DNC). However, in the enamel-delayed restoration group (EDR) bonding to enamel could not reach the level of the enamel-negative control group (ENC). Similar to our findings, in an *in situ* study Bittencourt et al. (7) detected that post-bleaching delay of the restorations for 7 or 14 days could not improve resin-enamel bond strength to the level of the enamel-control group, contrary to that observed in 21 days of restoration delay group. However, post-bleaching delay of the restorations for 7, 14, or 21 days could improve bonding to dentin to the level of the dentin-control group.

All antioxidant groups (ESA/DSA, EGT/DGT, ER/DR, EGTR/DGTR) exhibited higher mean SBS value and lower adhesive type failures compared to their relevant positive control groups (EPC/DPC), in which restorations were applied immediately after bleaching. Therefore, our first hypothesis stating that all experimental antioxidant protocols applied after bleaching would improve enamel-resin or dentin-resin shear bond strength, compared to the positive control was accepted.

Among the experimental antioxidant protocols applied to the bleached dentin (DSA, DGT, DR, DGTR), no significant difference was observed in terms of improving SBS. However, of all antioxidant protocols applied to the bleached enamel, the green tea and rosemary combination protocol (EGTR) was found to be less effective in improving SBS, comparing to the sodium ascorbate protocol (ESA). Additionally, contrary to other antioxidant protocols, green tea and rosemary combination protocol failed to enhance bonding to enamel/dentin in EGTR/DGTR groups to the level of relative delayed restoration groups (EDR/DDR). Therefore, our second hypothesis stating that there would be no difference between the antioxidant protocols in terms of improving bond strength to bleached enamel/dentin was partially rejected.

Free radical scavenging/antioxidant efficacy of sodium ascorbate on tooth structures has been well-documented (13). However, controversial results also exist (22,23), probably due to the variations in the experimental design of the studies. Various factors associated with the antioxidant protocol, e.g. the type/concentration of the antioxidant, the method/duration of the application, etc. can make an impact on the penetration capacity of the bioactive antioxidant

Table 5. Mean, standard deviation, minimum, maximum SBS data (in MPa) and fracture types of the dentin groups

Dentin groups (n=20)	SBS data (MPa)	Fracture types		
	Mean (±SD)*	Adhesive n, (%)	Cohesive n, (%)	Mix n, (%)
DNC	12.75 (±1.78)^{A}	13 (65%)	2 (10%)	5 (25%)
DPC	3.73 (±1.71)^{B}	20 (100%)	0 (0%)	0 (0%)
DDR	11.24 (±1.94)^{AC}	15 (75%)	0 (0%)	5 (25%)
DSA	10.52 (±2.95)^{ACD}	16 (80%)	0 (0%)	4 (20%)
DGT	10.17 (±2.56)^{CD}	17 (85%)	0 (0%)	3 (15%)
DR	9.38 (±2.89)^{CD}	17 (85%)	0 (0%)	3 (15%)
DGTR	8.57 (±2.68)^{D}	18 (90%)	0 (0%)	2 (10%)

*Significant differences between means are characterized by different uppercase letters ($p<0.05$).

DNC: Dentin negative control groups, DPC: Dentin positive control groups, DDR: Dentin delayed restoration groups, DSA: Dentin sodium ascorbate groups, DGT: Dentin green tea groups, DR: Dentin rosemary groups, DGTR: Dentin green tea & rosemary combination groups, SD: Standard deviation, n: Number

constituent(s) through oxidized enamel/dentin. This finally determines the free-radical scavenging effect obtained on the tooth structures.

The duration of the SA application in studies vary between 1 min to 40 h (13). Lai et al. (2) suggested that the duration of the antioxidant application should be at least one-third of the duration of the bleaching treatment. Türkün and Kaya (3) showed that a 10 min treatment duration was sufficient to increase post-bleaching enamel-resin bond strength, provided that the SA solution was applied actively to the bleached tooth surfaces. Park et al. (12) reported that when applied passively, the penetration of SA to tooth structures would occur quite slowly and take time due to the simple diffusion mechanism. Several other researchers also stated that by applying actively and refreshing continuously, the antioxidant effect of SA on bleached tooth surfaces could be enhanced (24,25). In another study, Freire et al. (14) revealed that the reaction of sodium ascorbate with the tooth surface reached its peak within 1 min and then gradually decreased. Researchers emphasized that the frequency of the SA application was more critical than the total application duration. In light of these findings, we applied SA to the bleached enamel/dentin surfaces actively and kept refreshing the solution once in per min throughout the application for 10 min.

The free radical scavenging effect of green tea extract on bleached enamel has been shown previously (4,6,17), which is in line with our results. Green tea is a rich source of catechins [epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC)], and flavonols. The strong antioxidant activity of green tea has been associated with its high content of catechin and flavonol, which can neutralize free radicals by donating hydrogen from hydroxyl groups in their structure (26,27).

Despite its well-documented antioxidant activity in the literature (28,29), interestingly, only a single *in vitro* study has investigated the free radical scavenging potency of rosemary on the oxidized tooth structure. In this study, Suneetha et al. (5) comparatively evaluated the rosemary-derived natural antioxidant solution (at an unspecified concentration) with the 10% sodium ascorbate solution on the enamel bleached with 10% carbamide peroxide. The researchers reported that both the rosemary-derived natural antioxidant protocol and SA showed satisfactory results in improving compromised resin-enamel SBS after bleaching. In another study, Prasansuttiporn et al. (30) comparatively investigated the antioxidant effect of 10% sodium ascorbate solution with 100 µM rosmarinic acid on dentin oxidized by NaOCl irrigation. Rosmarinic acid, which is a phenolic compound isolated from rosemary, was found to be more effective in improving bonding to dentin compared to SA protocol.

Nine different phenolic compounds with antioxidant activity, carnosol, carnosic acid, rosmanol, rosmadial, epirosmanol, isorosmanol, rosmarinidiphenol, rosmariquinone, and rosmarinic acid, were isolated from rosemary extracts (28). Richheimer et al. (29) reported that among all phenolic components of rosemary, carnosic acid had the greatest antioxidant potency, which was three times greater than carnosol and seven times greater than the synthetic antioxidants, BHT and BHA.

Several researchers have shown that the green tea extracts, combined with rosemary (18) or the other plant extracts (19,20) in the accurate concentrations, could create synergistic antioxidant interaction. Contrary to these findings obtained in the food industry research, synergistic antioxidant interaction could not be achieved in the current study, by combining green tea and rosemary antioxidant solutions at a ratio of 1:1.

Synergistic antioxidant interaction depends on combining the appropriate components in the accurate concentrations. The same components that produce a synergistic effect at a certain concentration may produce an antagonistic effect when combined in the other concentrations (16). As a limitation of this study, only a single antioxidant combination protocol was included in the experimental design. Further research is necessary to explore the possible antioxidant synergism between different individual plant extracts and/or isolated bioactive compounds in alternative combinations.

Numerous factors related to the complex nature of the plant extracts e.g. the region where the plant grows, the plant part used to obtain the extract, the extraction method of the plant, the types/concentrations of the phenolic compounds in the composition of the extract, etc. can have an impact on gathering different results from the antioxidant

activity studies. Plant extracts do not consist of a pure compound but contain many different antioxidant compounds with different molecular weights. Therefore, when plant extracts are employed for the preparation of the antioxidant solution, the molecular weight of the final solution cannot be determined (16,31). Nevertheless, studies as such, present indirect but useful information on the penetration capacity of the antioxidant compounds through oxidized tooth structures, by reflecting the re-increase in bonding occurred due to the neutralization of the free radicals.

As a low-cost preliminary *in vitro* research method, plant extract studies contribute and guide the pharmaceutical industry. Natural plant extracts and plant-derived compounds have been investigated for several therapeutic properties that could be beneficial in maintaining oral/dental health, such as wound healing, anti-cariogenic, antibacterial, anti-inflammatory, or antioxidant (31). The effects of green tea in decreasing dentin loss caused by erosion (32) alleviating halitosis (33), reducing periodontal inflammation (34), or preventing cavities (35) has been explored in literature. Similarly, the antibacterial effect of rosemary against cariogenic streptococci was also reported (36). Lately, several other therapeutic properties associated with plant extracts and/or isolated bioactive compounds, such as collagen-stabilizing, anti-MMP, or dentin modification potential (37,38) have raised great attention in adhesive dentistry.

Conclusion

Various pharmaceutical drugs/oral care products have been fabricated from the natural active ingredients of the plants or their synthetic derivatives produced under laboratory conditions to date. However, a gold standard antioxidant protocol or a commercially/globally available product with predictable and reproducible therapeutic results could not be generated for clinical use yet. Further studies are needed to explore the therapeutic potential of the different plant extracts/bioactive compounds either alone or combined at the proper proportions.

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Ethics

Ethics Committee Approval: This study does not require ethics committee approval.

Informed Consent: The patient is not included in this study.

Authorship Contributions

Concept: S.G., N.A.Y., Design: S.G., N.A.Y., Supervision: S.G., N.A.Y., Fundings: S.G., N.A.Y., Materials: S.G., N.A.Y., Data Collection or Processing: S.G., N.A.Y., Analysis or Interpretation: S.G., N.A.Y., Literature Search: S.G., N.A.Y., Critical Review: S.G., N.A.Y., Writing: S.G., N.A.Y.

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