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Effect of Pomegranate and Banana Extracts on Microshear Bond Strength of Resin Composite to Bleached Bovine Enamel: An In-Vitro Study

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ABSTRACT

Purpose: To evaluate the effect of pomegranate and banana extracts (natural antioxidants) versus ascorbic acid (synthetic antioxidant) prepared in 10% and 15% concentrations on microshear bond strength (μSBS) to bleached enamel. **Materials and Methods:** A total of 56 bovine incisors were selected for the current study. Forty teeth were used for microshear bond strength and divided into 5 groups according to antioxidant type; Group 1: No bleaching + No antioxidant (negative control), Group 2: Bleaching + No antioxidant (positive control), Group 3: ascorbic acid, Group 4: pomegranate extract and Group 5: banana extract. Groups 3,4 and 5 were further divided into 2 subgroups according to concentration of antioxidant: 10% and 15%. Bleaching gel was applied according to manufacturer instructions. Antioxidants were applied for 10 min. Composite specimens were prepared and tested for μSBS using universal testing machine. Sixteen bovine incisors were prepared and tooth-restoration interfaces were assessed using SEM. Data were tabulated and statistically analyzed. **Results:** Negative control and 15% ascorbic acid recorded the highest mean μSBS values followed by 15% and 10% pomegranate groups. No significant difference was reported between 10% ascorbic acid and 15% banana. Lowest mean values were obtained with 10% banana and positive control groups with no significant difference between them. **Conclusions:** Pomegranate in both concentrations and 15% banana extract could partially reverse compromised bond strength. Use of 15% ascorbic is able to totally regain bond strength to bleached enamel. Banana extract in low concentration is not beneficial in improvement of bond strength after bleaching.

KEYWORDS

Antioxidants, Microshear bond strength, pomegranate, banana extract, ascorbic acid.

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INTRODUCTION

The increasing demand for teeth whitening seeking for a brighter smile has been dramatically increased in the last few decades. Teeth bleaching is the most conservative and non-invasive line of treatment used for teeth whitening based on a complex oxidation reaction [1]. After bleaching, shade changes of existing esthetic restoratives are greatly expected which are usually associated with restoration replacement to achieve an acceptable color match[2]. Hydrogen peroxides and carbamide peroxides are the most commonly used agents for home and in-office bleaching. Although those agents reach satisfactory esthetic results, they have an adverse effect on bonding of resin composite to bleached enamel[3].

Bleaching agents have strong oxidizing properties. They can easily decompose to form oxygen free radicals which are able to penetrate tooth tissues and promote breakdown of pigments into minor molecular chains, resulting in whitening effect[4]. Residual oxygen present after tooth bleaching results in compromised bond strength due to inhibition of resin polymerization and hence, immediate bonding after bleaching is not clinically advisable [1].

To overcome diminished bond strength after bleaching, several techniques have been applied such as removal of superficial layer of enamel, postponing of bonding procedure for a period of time ranging from 24 hours to 4 weeks, treating enamel with alcohol or use of antioxidants [3,5]. Application of antioxidants after bleaching has been conducted with the aim of improving the compromised bonding of resin-based restoratives to enamel, reducing the risk for bond failure and enabling the immediate application of resin composite restoration by eliminating the time delay after bleaching [6,7].

Ascorbic acid is a powerful synthetic antioxidant that is considered the gold standard. The sodium salt of ascorbic acid (L-ascorbic acid) has been the most commonly used antioxidant on bleached tooth tissues [8]. Use of ascorbic acid has some drawbacks due to its instability under certain environmental conditions such as temperature, air, light, pH and humidity, which could negatively affect its antioxidant potential [9].

On the other hand, there are numerous natural antioxidants such as grape seed extract, green tea, tomato seed extract, pomegranate extract and banana extract which are safe, efficient, available and economical. The need for more stable and safe antioxidants has motivated researchers to pay attention to natural antioxidants of a plant origin that provide multiple therapeutic benefits with minimal or no side effects [10].

Pomegranate consumption has been increased due to its multiple functional and nutritional benefits. Pomegranate has many bioactive compounds, especially those belonging to the phenolic compound family such as tannins and anthocyanins [11]. Antioxidant activity is directly related to phenolic and flavonoid content [12]. Researchers found that banana peel extract is rich in bioactive compounds like flavonoids and polyphenols with multiple medical benefits as their high free radical scavenging activity [13,14]. Previous studies reported an antioxidant property of banana extracts used as medical treatment of various diseases such as cancer, anemia, ulcers, diabetes, burns, diarrhea, inflammation, cough and snakebite [12,15]. However, there are no studies conducted to investigate the antioxidant effect of banana extract on bond strength to bleached enamel. In addition, activity of antioxidants is greatly affected by their concentration, form and duration of their application[16].

Therefore, this study was conducted to investigate the effect of pomegranate and banana extracts in different concentrations on microshear bond strength of resin composite to bleached enamel in comparison to ascorbic acid.

MATERIAL AND METHODS

Preparation of pomegranate and banana peel extracts

Pomegranate (Punica granatum L.) and banana (Musa acuminata) peel extracts were prepared for the current study. Peels of each fruit were separated and thoroughly washed under running water. They were dried at room temperature for two weeks and ground into fine powder with a mechanical blender and sieved with a mesh. The dried powder of pomegranate and banana peels were extracted by 95% methanol using percolation method at room temperature according to method described by *Mohamed et al., 2016*[17]. The dried powder of both plants was placed in glass separating funnel and the solvent was added and kept for three days at room temperature. This procedure was repeated four times. Each extract was evaporated under reduced pressure at rotary evaporator (Heidolph, Germany) at 40ºC till dryness then were kept in a refrigerator until use.

Preparation of different concentrations of antioxidants

A total of 10 gm and 15 gm of pomegranate and banana extracts were dissolved in 100 ml of ethanol to obtain 10% and 15 % concentrations of both extracts. Regarding ascorbic acid, 10 gm and 15 gm of ascorbic acid were dissolved in 100 ml of distilled water to obtain 10% and 15% concentrations.

Determination of total phenolic and flavonoid content of pomegranate and banana extracts

For determination of total phenolic and flavonoid content, 10 mg of each extract was dissolved in 80% ethanol. Phenolic compounds were determined according to the colorimetric method described by *Swain and Hillis, 1959*[18]. Folin-Denis reagent was prepared using multiple constituents. Preparation was done by mixing 100 gm of sodium tungstate with 25 gm of sodium molybdate in addition to 700 ml of water, 50 ml of 85% phosphoric acid and 100 ml of concentrated hydrochloric acid in 1.5 L conical flask. The conical flask was connected to a reflux condenser and then gentle boiling was done for 10 hours. The mixture was left to cool till reach room temperature. After cooling, 150 gm of lithium sulphate, 50 ml of water and few drops of liquid bromine were added. This mixture was boiled for 15 min without condenser in order to get rid of excess bromine, left to cool, diluted to 1 liter and then filtered. Saturated solution of sodium carbonate was prepared by adding 35 gm of anhydrous sodium carbonate to 100 ml of water at high temperature (70 -80ºC), left to cool, kept overnight and then filtered. An aliquot of the prepared extract and 0.5 ml of Folin-Denis reagent were thoroughly mixed in a dry test tube. The tube was well shaked for 3 min, then 1.0 ml of saturated Na_2CO_3 solution was added and thoroughly mixed, after that 3 ml of distilled water was added. After one hour, quantities were determined by reading the developed blue color at 725 nm.

Total flavonoid content was determined according to the method described by *Zhuang et al. 1992* [19]. A 0.5 mL aliquot of sample solution was thoroughly mixed with 2 mL of distilled water and then with 0.15 mL of $NaNO_2$ (5%) solution. After 6 min, 0.15 mL of $AlCl_3$ (10%) solution was added and left for further 6 min. Thereafter, 2 mL of NaOH (4%) solution was added to the mixture followed by distilled water to reach a final volume of 5 mL. This mixture was properly mixed and left for 15 min. Absorbance of the mixture was taken at 510 nm.

Determination of total antioxidant activity of different antioxidants

Antioxidant activity of ascorbic acid, pomegranate extract and banana extract were measured following multiple steps as follow:

1. Initial screening test: solutions of the tested antioxidants were prepared in two concentrations of 1000 and 100 μg/mL in 80% ethanol

aiming to identify their inhibitory concentration 50 (IC50) range.

2. C50 determination: antioxidants that recorded more than 50% inhibition of free radicals in any concentration of the initial screening test were diluted to provide 5 serial concentrations.
3. Ascorbic acid standard preparation: 100μM concentration of ascorbic acid was prepared in methanol from which 7 serial concentrations were prepared including 5, 10, 15, 20, 30, 40 and 50 μM.
4. DPPH Assay: DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was tested based on the method described by *Boly et al 2016* [20] to determine number of free radicals. A 100μL of the prepared DPPH reagent (0.1% in methanol) were added to 100μL of the tested antioxidant in 96 wells plate and incubated for 30 min at room temperature in a dark environment. Reduction in DPPH color intensity was investigated at 540 nm.

Micro plate reader analysis

The results were recorded using micro plate reader FluoStar Omega. 1.2.2. Data analysis. Data was analyzed using Microsoft Excel® and the IC50 value was calculated using Graph pad Prism 5 by converting the prepared concentrations to their logarithmic value and selecting nonlinear inhibitor regression equation (log (inhibitor) vs. normalized response – variable slope equation) [21].

Ethical consideration

This study was approved from the Research Ethics Committee of the Faculty of Dental Medicine for Girls, Al-Azhar University. The research final code is REC-OP-21-09.

Sample size calculation

A power analysis was determined to have adequate power to apply a statistical test of the null hypothesis that there is no difference between the tested groups regarding bond strength. By adopting an alpha and beta levels of (0.05) i.e. power=95% and an effect size (f) of (0.562) calculated according to the results of a previous research [22]. The total predicted sample size (n) was found to be 80 samples. Sample size calculation was done using G*Power version 3.1.9.7 [23].

Teeth selection, grouping and preparation

A total of 56 bovine incisor teeth were selected for the current study (40 teeth for microshear bond strength testing and 16 teeth for scanning electron microscopic observation). Forty teeth were used for microshear bond strength test and were randomly divided into 5 main groups according to type of antioxidant used as follow; Group 1: No bleaching + No antioxidant (negative control) (n=5), Group 2: Bleaching + No antioxidant (positive control) (n=5), Group 3: ascorbic acid (n=10), Group 4: pomegranate extract (n=10) and Group 5: banana extract (n=10). Groups 3,4 and 5 were further divided into 2 subgroups (n=5) according to concentration of antioxidant; 10% and 15%.

Teeth were thoroughly washed under running water, scaled to remove any soft or hard deposits and stored in distilled water at 4°C for not more than one month. The root apices were cut under copious water coolant using a diamond disc (1Disco de Diamante C01/ 220, 0.20 mm) in a straight hand piece. Teeth crowns were embedded in self-cured acrylic resin (Acrostone, Egypt) in molds (2 cm internal diameter and 1.2 cm height) with their labial surfaces facing upward. Sandpaper discs of 300 grit were used for flattening of enamel surface and 600 grit were used for smoothening of enamel surface to achieve a standardized smear layer.

Table(1) *List of the materials used in the study their brand name, description, composition, manufacturer and lot number*

Brand Name and description	Composition	Manufacturer	Lot number
The smile strong (Bleaching agent)	38%hydrogen peroxide, nitrate potassium, sodium fluoride, methacrylate, not hazardous additives	Health & beauty oasis srl corso Milano, 83,35139 padova Italy www.unicagroup.it	RR1720335
Tetric N -Flow (Flowable composite)	36wt. % dimethacrylates (including TEGDMA) 63wt.% fillers and 1 wt. %initiators, pigments and stabilizers. Inorganic fillers are 39 vol. %. The particle size range of inorganic fillers is 40-3000 nm.	Ivoclar vivadent AG 9494 schaanl/Liechtenstein www.ivoclarvivadent.com	YZ1212
Tertic N-Bond (Universal adhesive)	Phosphoric acid acrylate, HEMA, Bis-GMA, urethane dimethacrylate, ethanol, initiators, stabilizers and film-forming agent	Ivoclar vivadent AG 9494 schaanl/Liechtenstein www.ivoclarvivadent.com	Y38643
N-Etch. (Etchant gel)	37% phosphoric acid	Ivoclar vivadent AG 9494schaanl/Liechtenstein www.ivoclarvivadent.com	Y39063
Ascorbic acid (Synthetic antioxidant)	L-Ascorbic Acid extrapure AR,99.7%	Sisco Research Laboratories Pvt. Ltd. 608, B Wing, Sateline Gazebo, Andheri Ghatkopar. Mumbai-400009 India www.srlchemicals.com	23006 0149100

Bleaching procedure

A layer of the bleaching gel was applied on the labial surface of each tooth using a microbrush (Blue Bobcare Micro Applicators), left for 20min following the manufacturer's instructions, washed thoroughly under running water and then air dried. This procedure was repeated with total of 3 consecutive applications as described by the manufacturer.

Antioxidant application

After bleaching procedure, the groups assigned for treatment with antioxidant solutions were treated using ascorbic acid, pomegranate extract and banana extract in both 10% and 15% concentrations. Antioxidants were applied to bleached enamel using microbrush, left for 10 min, washed under running water and gently air dried.

Bonding procedure and packing of resin composite

The specimens were etched using 37% phosphoric acid etchant gel for 15 sec according to the manufacturer's recommendations, rinsed for 15 sec and air dried. After etching, 2 coats of the adhesive were applied using a disposable microbrush and rubbed for 20 sec then gentle air streaming was done for 5 sec to ensure complete evaporation of the solvent.

Before curing of the adhesive, two rubber microtubes (Tygon) of 0.8 mm internal diameter and 1 mm height were placed on the flat enamel surface of the tooth to act as molds for the flowable composite specimens. Adhesive was light cured for 20 sec using LED light curing device (Woodpecker iLED curing Light, Borkstrasse10, 48163 Muenster, Germany) with light intensity 2300 Mw/cm^2 which

was periodically checked after each group using a radiometer (Ledex, LED Radiometer).

Flowable composite was injected inside the microtubes, covered by celluloid strip and light cured for 20 sec, using the same light curing device. On each bovine tooth, 2 resin composite specimens were applied with a total of 10 specimens for each experimental group. The rubber microtubes were removed by sectioning it in longitudinal direction using a sharp lancet. All debonded specimens were excluded. Specimens were stored in deionized water for 24 h at 37°C in an incubator (Laboratory incubator CLN32) till testing.

Microshear bond strength testing

Each bovine tooth with the bonded resin composite specimens was secured to the lower fixed compartment of the universal testing machine (Lloyd LR5K, Lloyd Instruments Ltd., and Hampshire, UK). Testing was done at 0.5 mm/min cross head speed using a thin ligature orthodontic wire loop attached to the upper compartment of the universal testing machine. The load was applied at the enamel-composite interface till debonding. Load was recorded automatically using the machine operating software, stress was calculated and presented in MPa (Nexygen software, version4.6, Lloyd Instruments Ltd., Hampshire, UK).

Scanning electron microscopic (SEM) observation

A total of 16 bovine incisors (two teeth for each group) were selected for observation of tooth-restoration interface using scanning electron microscope. Teeth were grouped, prepared, bleached and treated with antioxidants in the same manner as previously mentioned. Resin composite specimens (2mm thickness × 3mm diameter) were prepared on the labial surfaces of teeth using mold with the same dimensions. The specimens were sectioned into two halves using diamond disc under copious water coolant to expose the tooth-restoration interface. The cut surfaces were ground with 600 grit sandpaper disc and then rinsed using distilled water. The flat polished surfaces were treated with 6% citric acid for 20 sec and rinsed with water. The specimens were then immersed in 5% sodium hypochlorite for 2 min, after which they were copiously rinsed with distilled water [24]. The specimens were dehydrated through immersion in 99% ethyl alcohol for 30 sec and then left to dry. The sectioned parts were kept in labeled containers according to their corresponding groups. Specimens were vacuum dried and gold sputter coated (S150 A Sputter coater, UK), after which the samples were imaged using scanning electron microscope (Quanata Feg 250) at 2000X magnification.

Statistical analysis

Data were presented as mean, standard deviation (SD) and confidence intervals. Data were explored to determine their normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test found that data were normally distributed (parametric data), therefore one way analysis of variance (ANOVA) test was done followed by Tukey's post hoc test for comparison between groups. Independent t test was done to compare values obtained from different concentrations (10% and 15%) within the same antioxidant. Level of significance was set at $p \leq 0.05$. Statistical analysis was done with SPSS 18.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

RESULTS

Results of total phenolic and flavonoid content of pomegranate and banana extracts

Determination of total phenolic and flavonoid percentage showed that total phenols percentage of pomegranate extract is 11.155 and that of banana is 4.710 while for total flavonoids percentage, pomegranate extract recorded 1.012 and banana extract recorded 0.258.

Results of total antioxidant activity of all different antioxidants

Assessment of antioxidant activity (DPPH free radical assay) of all tested antioxidants revealed that ascorbic acid recorded 24.42±0.87, pomegranate extract recorded 29.68±1.70 and banana extract recorded 300.6±11.31. High readings indicate the presence of large number of free radicals which subsequently demonstrate low antioxidant activity.

Results of microshear bond strength test

ANOVA test revealed significant difference between different experimental groups (p=0.000). Tukey's post hoc test demonstrated that the highest microshear bond strength mean values were recorded by no bleaching + no antioxidant (negative control) group (19.60±2.06) and bleaching + 15% ascorbic acid group (20.06±2.43) with no significant difference between them. This was followed by bleaching + 15% pomegranate group (17.33±1.35) then bleaching + 10% pomegranate group (12.69±1.41). No significant difference was recorded between bleaching + 15% banana group (9.21±1.44) and bleaching +10% ascorbic acid group (9.18±1.15). The lowest mean values were recorded by bleaching + no antioxidant (positive control) group (5.2±.65) and bleaching + 10% banana group (4.74±1.28) with no significant difference between them (Table 2).

Table (2): *Descriptive statistics and test of significance of microshear bond strength (MPa) in different experimental groups*

	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max	F	P
				Lower Bound	Upper Bound				
No bleaching + no antioxidant (Negative control)	19.60[a]	2.06	0.65	18.13	21.07	17.38	23.96	155.8	.000*
Bleaching + No antioxidant (positive control)	5.20[e]	0.65	0.21	4.73	5.66	4.06	6.19		
Bleaching +10% Ascorbic Acid	9.18[d]	1.15	0.37	8.35	10.01	7.28	10.55		
Bleaching + 15% Ascorbic Acid	20.06[a]	2.43	0.77	18.32	21.80	16.81	23.64		
Bleaching+ 10% pomegranate	12.69[c]	1.41	0.44	11.68	13.69	10.72	14.97		
Bleaching+ 15% pomegranate	17.33[b]	1.35	0.43	16.37	18.30	15.30	19.76		
Bleaching +10% Banana	4.74[e]	1.28	0.40	3.82	5.65	3.35	6.81		
Bleaching + 15% Banana	9.21[d]	1.44	0.48	8.10	10.32	6.42	11.34		

*Tukey's post hoc test: Within the same comparison (concentration), means with the different superscript letter are significantly different. Significance level p≤0.05, * significant*

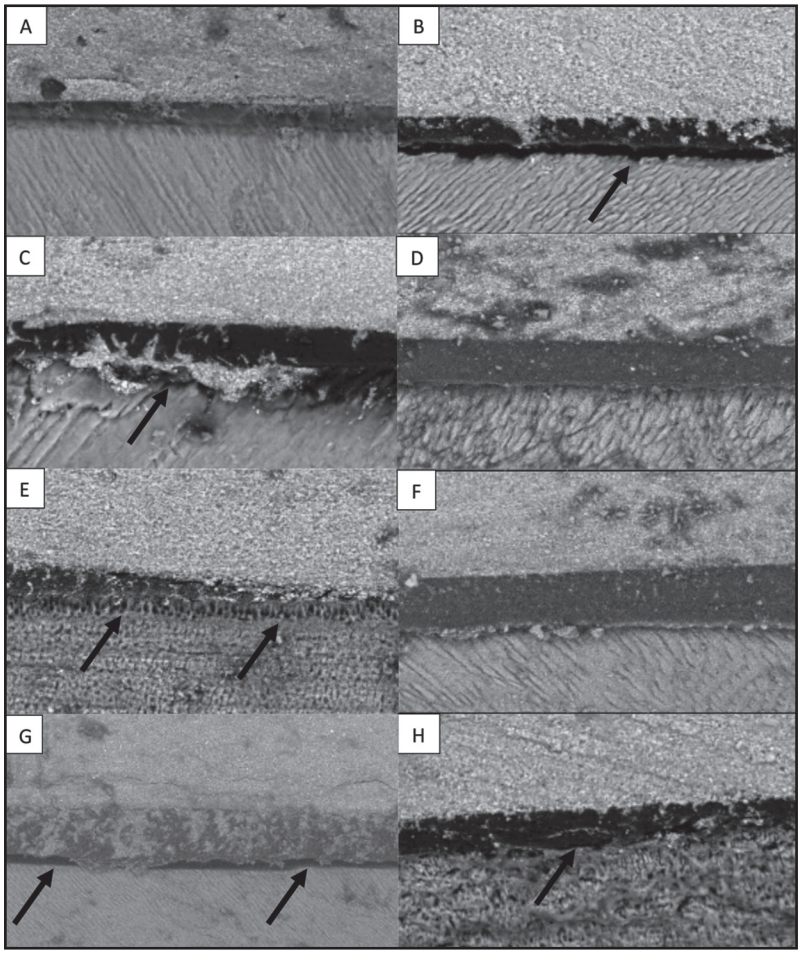
Results of different concentrations of antioxidants revealed that 15% concentration recorded significantly higher microshear bond strength mean value than 10% concentration in all tested antioxidants (p=0.000).

Table (3): *Effect of different concentrations of antioxidants on microshear bond strength (t test)*

Groups	Conc.	Mean	Std. Dev	Paired difference				t	P
				Mean	Std error	C.I. lower	C.I. upper		
Bleaching + ascorbic acid	10%	9.18	1.15	10.88	.85	9.09	12.67	12.78	.000*
	15%	20.06	2.43						
Bleaching + pomegranate	10%	12.69	1.41	4.65	.62	3.35	5.94	7.54	.000*
	15%	17.33	1.35						
Bleaching + banana	10%	4.74	1.28	4.48	.63	3.15	5.80	7.14	.000*
	15%	9.21	1.44						

*C.I.: 95% confidence interval, Significance level p≤0.05, * significant*

Scanning Electron Microscopic (SEM) observation



Figure(1) SEM images at 2000 X magnification showing enamel-composite interfaces in different experimental groups; (A) Group 1 (no bleaching + no antioxidant) showing a uniform gap free adhesive junction, (B) Group 2 (bleaching + no antioxidant) showing wide interfacial gaps along the adhesive interface, (C) Group 3 (10% ascorbic acid) showing non homogenous adhesive junction with entrapped small voids, (D) Group 4 (15% ascorbic acid) showing uniform intact adhesive junction, (E) Group 5 (10% pomegranate extract) showing micropores along the whole length of the adhesive layer, (F) Group 6 (15% pomegranate extract) showing homogenous gap free adhesive junction, (G) Group 7 (10% banana extract) showing gap formation along the adhesive interface, (H) Group 8 (15% banana extract) showing homogenous layer with evidence of minimal interfacial gap.

DISCUSSION

This study investigated the effect of pomegranate and banana extracts (natural antioxidants) versus ascorbic acid (synthetic antioxidant) prepared at 10% and 15% concentrations on microshear bond strength to bleached enamel.

Nowadays, teeth bleaching is one the most common modalities used for treatment of discolored teeth. It is a conservative treatment that permits a successful aesthetic outcome with minimal expenses [25,26]. Despite of the satisfactory esthetic results obtained after teeth bleaching, some drawbacks has been observed as morphological alterations of teeth, increased surface roughness, reduced microhardness in addition to compromised bond strength of composite resin to bleached enamel [27].

Bond strength is compromised after bleaching due to entrapment of oxygen, hydroxyl and per-hydroxyl ions in tooth structure which result in inhibition of resin polymerization [28]. The hydroxyl ions in the apatite lattice are replaced by peroxide ions during bleaching resulting in formation of unstable lattice structure. Those peroxide ions are decomposed gradually within 2 weeks after bleaching and the hydroxyl ions regain its position in the apatite lattice [29]. Use of antioxidants is recommended after teeth bleaching to regain bond strength and allow immediate bonding without need for time delay. In the current study, pomegranate and banana extracts were used versus ascorbic acid to investigate their effect on microshear bond strength.

Bovine teeth were utilized in the current study to assess the microshear bond strength of resin composite to bleached enamel. They have the advantages of being available, free of caries and have large surface area which facilitates application of more than one specimen [30,31]. Previous research reported no significant difference in mean bond strength values between bovine and human teeth [32] thus, use of bovine teeth is appropriate and acceptable.

Antioxidant activity of pomegranate peel extract versus seed extract was investigated and the results revealed higher antioxidant capacity in peel extract[33]. Also, banana peel extract recorded higher total phenolic content and total antioxidant activity than that extracted from banana pulp [34]. Based on these studies, pomegranate and banana peel powder extracts were prepared and used in the current study. Antioxidant solutions were applied for 10 min in the present study. This duration is applicable in clinical situations and revealed significant improvement of microshear bond strength to bleached enamel [16,26].

Microshear bond strength was used in the current study to determine the bond strength of resin composite to bleached enamel. It is considered one of the popular and accurate methods. It is characterized by simplicity of specimens' preparation and preparing multiple specimens on the same substrate[35].

Regarding results of microshear bond strength as reported in table (2), there was statistically significant difference between tested antioxidants. It was found that application of 15% ascorbic acid exhibited the highest mean bond strength value among tested antioxidants with no significant difference compared to no bleaching (negative control) group.

Antioxidants are compounds that have free radical scavenging potential through one of two mechanisms. The first mechanism is that it donates a hydrogen ion to the free radical (ArOH) and subsequently, itself becomes a radical: $R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet}$. The second mechanism occurs via giving an electron to the free radical becoming itself a radical cation: $R^{\bullet} + ArOH \rightarrow R^{-} + ArOH^{\bullet+}$ [36].

Antioxidant activity is related to molecular structure specially number of hydroxyl groups [36]. Ascorbic acid contains 4 OH groups that can donate hydrogen to the oxidizing system with scavenging effect of free radicals. This allows polymerization of adhesive resin without premature termination [37]. High antioxidant activity of ascorbic acid was previously reported [9]. It was also confirmed by

total antioxidant activity testing investigated in the current study.

Another explanation for the high bond strength results of 15% ascorbic acid might be due to its acidity. Ascorbic acid is highly acidic with pH 1.8 [38] which might cause etching of the bonding substrate with enhancement of micromechanical retention and improvement of bond strength [39]. Scanning electron microscopic images of 15% ascorbic acid showed intact gap free tooth-restoration interface (fig. 1D). This result was in agreement with previous researches [40,41].

Regarding different concentrations of ascorbic acid (table 3), 15% concentration showed a significantly higher mean value than 10% concentration. It was reported in a previous study [42] that the use of 10% sodium ascorbate led to formation of limited resin tags and hybrid layer. Although, they were continuous and homogeneous across the adhesive interface but, they were still not comparable to the unbleached enamel. This might give an explanation why 10% ascorbic acid was not able to totally regain reduced microshear bond strength unlike 15% ascorbic acid which was effective to regain microshear bond strength comparable to negative control group. This finding was in accordance with *Tabatabaei et al, 2011 and Subramonian et al, 2015* [43,44]. This was further confirmed by scanning electron microscopic image of 10% ascorbic acid (fig. 1C) which showed non-homogeneous adhesive layer with small voids entrapped within the adhesive junction.

Regarding results of pomegranate, it was found that both concentrations (10% and 15%) significantly improved microshear bond strength comparable to the positive control group, although they were significantly lower than unbleached group. This finding might be attributed to chemical structure of pomegranate as it is composed of high concentrations of phenolic and flavonoid content [45] which are directly correlated to its antioxidant potential [46]. Phenols act as reducing agents by donating electrons that react with the present free radicals and convert them to a more stable component with termination of free radical chain reaction [47].

The antioxidant activity of phenols is usually related to the presence and number of hydroxyl groups in their structure [37]. Pomegranate contains tannins and anthocyanins. The chemical structure of tannins [48] and anthocyanins [49] is rich in hydroxyl groups. This was also confirmed by the results of the present study which investigated high phenol and flavonoid content of pomegranate peel extract.

In addition, one of the constituents of pomegranate is ascorbic acid [48] which might further explain this result. This finding was in agreement with *Sharafeddin et al, 2015 and Fares et al, 2017* [25,50] who investigated the effect of pomegranate on bond strength to enamel after tooth bleaching and revealed satisfactory results.

In the current study, the use of 15% pomegranate extract showed significantly lower microshear bond strength results in comparison to 15% ascorbic acid. A previous study [25] revealed no significant difference between pomegranate and sodium ascorbate on bond strength of resin composite to bleached enamel. According to the results of total antioxidant activity of the current study, ascorbic acid recorded a slightly higher antioxidant activity (24.42±0.87) than pomegranate extract (29.68±1.7) with minimal difference between them. Sodium ascorbate is characterized by its neutral pH 7.4 [41] while ascorbic acid is highly acidic with pH 1.8 [38]. The superior performance of ascorbic acid in comparison to pomegranate might be related to it is high acidity which might improve micromechanical retention via etching of enamel substrate and subsequently bond strength as mentioned before.

Concerning the effect of different concentrations of pomegranate (table 3), 10% concentration demonstrated significantly lower mean microshear bond strength value in comparison to 15% concentration. Lower concentrations are usually

associated with lesser phenolic and flavonoid contents which subsequently result in lower antioxidant activity represented by lower microshear bond strength mean values. SEM image of 10% pomegranate (Fig. 1E) showed micropores along the adhesive interface.

Results of microshear bond strength reported that the use of 15% banana extract improved microshear bond strength in comparison to positive control group with no significant difference compared to 10% ascorbic acid (table 2). The lowest mean bond strength value was recorded by 10% banana extract which showed no significant difference comparable to positive control group. Total phenolic and flavonoid content and antioxidant capacity of banana varies according to its type [51]. According to results of the current study, banana extract demonstrated low total phenolic and flavonoid content and low antioxidant activity which indicates a minimal ability to eliminate residual oxygen and restore bond strength. This was further confirmed by the scanning electron microscopic images which showed gap formation along the adhesive interface with 10% banana concentration as shown in fig. 1G.

The current research is considered the first report about the antioxidant activity of banana extract on bond strength of resin composite to bleached enamel. It would be inspiring to assess effect of different concentrations, methods of extraction and forms. Also, temperature and related factor should be taken into consideration.

CONCLUSION

Within the limitations of the current study, it could be concluded that pomegranate in both concentrations and 15% banana extract could partially reverse compromised bond strength. Use of 15% ascorbic acid is able to totally regain bond strength to bleached enamel. Banana extract in low concentration is not beneficial in improvement of bond strength after bleaching.

RECOMMENDATION

Studies are recommended to assess the effect of different concentrations of pomegranate and banana extracts as well as the effect of different methods of extraction and forms on bleached enamel.

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This study did not receive any financial support or sponsorship.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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