Comparative evaluation of the effect of 10%, 20%, and 30% guava seed extract on reversing compromised resin bond strength after enamel bleaching in 120 min, 10 min, and 5 min: An in vitro study

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Abstract

Background: Bonding of composite with enamel and dentin gets compromised if the tooth is restored with composite immediately post bleaching. The application of antioxidants has shown to have a positive effect on it.

Aim: The aim of this study was to evaluate and compare the effect of guava seed extract solutions in various concentrations on the shear bond strength (SBS) of composite resin to bleached enamel when applied for 5 min, 10 min, and 120 min.

Materials and Methods: Fifty-five maxillary incisors were procured, and labial surfaces of 50 specimens were bleached with 37.5% hydrogen peroxide. These specimens were divided into 3 experimental groups: Group 1–3 comprising 15 specimens each (n = 15), and the remaining specimens were divided into two groups: Group 4 (positive control group) and Group 5 (negative control group). Groups 1–3 were further divided into 3 subgroups according to the application period of antioxidant: subgroups A, B, and C. Specimens were stored in distilled water for 24 h. SBS testing was done using the universal testing machine. Data were tabulated and subjected to statistical analysis using a three-way analysis of variance with post hoc Tukey’s test.

Results: Guava seed extract showed a complete reversal of the compromised bond strength, and promising results were seen with increased concentration.

Conclusion: The use of antioxidants effectively reversed the compromised bond strength of bleached enamel, and an increase in the concentration of antioxidants reduces the duration of its application.

Keywords: Antioxidants; bleaching; bond strength; composite resin; guava seed extract

INTRODUCTION

Discolored teeth are frequently encountered in day-to-day clinical practice and are quite challenging to manage. Bleaching has been one of the most noninvasive managements of the discoloration since it does not involve cutting or drilling of the hard dental tissue.[1] Bleaching is classified as vital and nonvital bleaching. Vital dental bleaching agents contain H₂O₂ (3%-40%) or carbamide peroxide (10%).[2] Lasers and lights are used to initiate the bleaching procedure resulting in chemical interaction that increases the release of hydrogen released from H₂O₂.[3]
H₂O₂ is an agent that decomposes and creates unstable free radicals when it diffuses into the tooth and causes oxidation. Many studies have shown that carbamide peroxide and H₂O₂ can negatively affect the bonding strength of the composite to the tooth structure.

Along with various advantages, bleaching has some adverse effects such as hypersensitivity, gingival irritation, micromorphological defects, and reduced enamel microhardness and also affects the bonding of enamel and dentin tissues when composite restoration is done immediately post bleaching. Taking the latter problem into account, i.e., compromised bonding of composite with enamel and dentin, various techniques are involved to resolve it. The various techniques include: (i) postponing any bonding procedure for 4 days to 4 weeks because waiting for 1–3 weeks has shown the decomposition of the peroxide ions, (ii) conditioning the bleached enamel with alcohol, (iii) removing the surface layer of enamel and employing adhesives containing organic solvents, and (iv) the use of antioxidants. The most effective treatment is the use of antioxidants such as 10% sodium ascorbate, Vitamin E, 2% sweet potato extract and proanthocyanidin postbleaching.

Guava seed extract is proanthocyanidin-derived fruit that has the highest antioxidant activity after star fruits. High antioxidant activity of guava might be responsible for the reversal of reduced bond strength. It has the free radical scavenging ability helping in preventing the oxidation process that causes the failure of bonding of composite with enamel and dentin.

The use of guava seed extract has shown to be effective in reversing the compromised bond strength of bleached enamel completely when used in the concentration of 10% when applied for 10 min. The study aims to evaluate and compare the effect of guava seed extract solutions in various concentrations on the shear bond strength (SBS) of composite resin to bleached enamel when applied for 5 min, 10 min, and 120 min. The null hypothesis of the study is that the increase in the concentration of the antioxidant does not reduce the duration of its application.

**MATERIALS AND METHODS**

Fifty-five extracted incisors were procured and disinfected by 0.5% chloramine and stored in 10% formalin. Smear layer was standardized by creating the smear layer by wet grinding with 600 grit SiC paper in enamel in the labial surface of the tooth.

Individual molds of tooth-colored self-cure resin were prepared over the specimens. Modeling wax was used as a separator and also to provide space for the antioxidant solution on the labial surface of the specimens. A customized cylindrical silver mold of 3 mm diameter and 5 mm height was fabricated.

**Preparation of guava seed extract [Figure 1]**

Soxhlet extraction method was used for the preparation of 10%, 20%, and 30% guava seed extract. 40 g of guava seeds were placed inside a thimble (thick filter paper pouch), which was kept in the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a distillation flask containing 40 ml of n-hexane as an extraction solvent. The solvent was then heated to reflux. The chamber containing the solid material was slowly filled with warm solvent as it is being heated up. When the Soxhlet chamber was almost full, the chamber was automatically emptied via a siphon side arm, with the solvent running back down to the distillation flask. This cycle was repeated many times over 5 h. During each cycle, a portion of the nonvolatile compound was dissolved in the solvent. After many cycles, the desired compound was concentrated in the distillation flask. After extraction, the solvent was removed by means of a rotary evaporator. 10 ml, 20 ml, and 30 ml of this extract were then diluted in 90 ml, 80 ml, and 70 ml of ethanol to make 10%, 20%, and 30% guava seed extract solution, respectively, and were stored in the tightly sealed bottle.

Labial surfaces of 50 specimens were bleached with Pola office one patient kit (SDI, Victoria, Australia) containing 37.5% hydrogen peroxide with four applications of 8 min each. The gel was then rinsed off with water. These specimens were divided into 5 groups and further divided into 3 subgroups according to the application period of antioxidants.

- **Group 1 (n = 15):** Treatment with 10% guava seed extract solution
- **Group 2 (n = 15):** Treatment with 20% guava seed extract solution
- **Group 3 (n = 15):** Treatment with 30% guava seed extract solution
- **Group 4 (n = 5):** Treatment with no antioxidant solution-positive control group
- **Group 5 (n = 5):** Control (unbleached specimens) – negative control group. Based on the application period of antioxidants, Groups 1–3 were further subdivided as:
  - **Subgroup A (n = 5):** Antioxidant treatment for 5 min immediately after bleaching
  - **Subgroup B (n = 5):** Antioxidant treatment for 10 min after bleaching.
  - **Subgroup C (n = 5):** Antioxidant treatment for 120 min after bleaching.

The antioxidant solutions were refreshed every 10 min in subgroup C. No antioxidant treatment was done for Group 4 and Group 5.
Then, the specimens were rinsed with water, and were bonded with composite resin. A customized cylindrical silver mold was placed on the labial surface of each specimen, and the composite resin was then placed in three increments to have a final mass of 3 mm diameter and 5 mm height. Etching, bonding, and composite buildup were done, each increment cured for 20 s. All specimens, after the composite buildup, were stored in distilled water for 24 h.

SBS testing was done with a universal testing machine at a crosshead speed of 1 mm/min [Figure 2]. Data were tabulated and subjected to statistical analysis using a three-way analysis of variance with post hoc Tukey’s test.

RESULTS

On intergroup comparison, Group 5 had significantly higher mean value (control group), followed by Group 3, and Group 4 (control group) had significantly lower mean value as compared to other groups/subgroups. On intergroup comparison of bond strength of Group 3, there was no significant difference among subgroup A, subgroup B, and subgroup C [Figure 3]. The order of the bond strength (mean value) of Group 3 was 4 < 3A = 3B = 3C > 5.

DISCUSSION

When composite restorations are applied immediately post bleaching, it affects the bonding of composite with bleached enamel and dentin. The application of antioxidants has shown to have an influence in reversing this compromised bond strength. There are various studies that advocate for the use of antioxidants in reversing the compromised bond strength.[16-18]

Guava seed extract having the high antioxidant property was used for the study. Along with the antioxidant property, guava seed extract has the antimicrobial and anticarcinogenic properties as well.[19,20] Guava seed extract in all the concentrations was capable of reversing the reduced bond strength following bleaching. Individual resin molds were prepared for adequate contact of antioxidants with the labial surface of the specimens. The guava seed extract was prepared from white guava seeds using the Soxhlet extraction method. This method is advantageous as it allows only one consignment of solvent to be recycled instead of several batches of warm solvent.

The extract was diluted using ethanol (alcohol) to make it 10%, 20%, and 30% concentration since the extract is immiscible in water. Studies have also demonstrated that the presence of alcohol may have helped in increasing the bond strength. Thus, the phenomenon observed may be system specific, not only due to antioxidant agent but also due to the presence of alcohol.

To determine the application of guava seed extract with various concentrations, i.e., 10%, 20%, and 30%, for reversing the reduced bond strength, various application periods (5 min, 10 min, and 120 min) were chosen for the current study. Hulas Gogia et al. have stated that the application of 10% guava seed extract for 10 min reverses the compromised bond strength to its baseline levels.[15]

The most frequently ignored guideline in the test protocol is the limitation of the bonding area. Hence, the mold of silver with a 3 mm internal diameter and 5 mm height was made.

In the present study, the mean SBS of Group 5 (control group) was statistically significant when compared to all the other groups that indicate better bonding if adhesive procedures are performed without bleaching. These findings corroborate with the results of previous studies.[21]
Hence, this process is deleterious during in vitro [22]. The duration of its application was rejected. The concentration of the antioxidant does not reduce the duration. Thus, the null hypothesis that the increase in SBS with 30% guava seed extract when applied for a shorter duration was made.

Complete reversal of compromised SBS was observed when the solution was refreshed every 10 min. Whereas, Group 2 showed the highest mean SBS in Group 2C. It could be because of the prolonged period of application time when the solution was refreshed every 10 min. Whereas, intragroup comparison of Group 3 showed a similar mean SBS in all the subgroups.

In Group 1, Group 2, and Group 3, the mean SBS is increased, indicating that the use of antioxidants on the surface of bleached enamel before restoration helps in reversal of compromised bond strength.[21] An increase in the concentration of guava seed extract increased the SBS. Complete reversal of compromised SBS was observed with 30% guava seed extract when applied for a shorter duration. Thus, the null hypothesis that the increase in the concentration of the antioxidant does not reduce the duration of its application was rejected.

**Figure 3:** Bar graph of intergroup and intragroup comparison of bond strength

It was also observed that immediate bonding to bleached enamel without application of antioxidant (guava seed extract) such as in Group 4 resulted in a significant decrease in the mean SBS when compared to all the other groups. This result indicates that bleaching of the enamel causes compromised bond strength between bleached enamel and composite resin.

Bleaching agent releases free radicals in the form of nascent oxygen and hydroxyl or perhydroxyl ions, when applied to the dental structure, and a high concentration of oxygen remains among the enamel prisms and in the dentin.[22] The dentin and dentinal fluid can act as peroxide- and oxygen-free radical reservoir and could persist until removed by pulpal microcirculation or be released later through surface diffusion.[23] Hence, this process is deleterious during bonding of the composite resin, as higher levels of peroxide or oxygen may be present in the bonding surface, inhibiting the polymerization and thus reducing the bond strength.

Intragroup comparison of Group 1 showed a similar mean SBS in Groups 1B and 1C. Intragroup comparison of Group 2 showed the highest mean SBS in Group 2C. It could be because of the prolonged period of application time when the solution was refreshed every 10 min. Whereas, intragroup comparison of Group 3 showed a similar mean SBS in all the subgroups.

In Group 1, Group 2, and Group 3, the mean SBS is increased, indicating that the use of antioxidants on the surface of bleached enamel before restoration helps in reversal of compromised bond strength.[21] An increase in the concentration of guava seed extract increased the SBS. Complete reversal of compromised SBS was observed with 30% guava seed extract when applied for a shorter duration. Thus, the null hypothesis that the increase in the concentration of the antioxidant does not reduce the duration of its application was rejected.

**CONCLUSION**

- Guava seed extract, when used in any concentration, improved the compromised bond strength.
- An increase in the concentration has shown to have improved SBS between bleached enamel and composite restoration even when applied for a shorter duration.
- Increased concentration has reduced the duration of the application time of guava seed extract.
- Further studies on the effect of increased concentration in the mechanical structure/microhardness of enamel are required.

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**Conflicts of interest**

There are no conflicts of interest.

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