*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*
*e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 22, Issue 7 Ser.4 (July. 2023), PP 42-51*
*www.iosrjournals.org*

# "The Effect Of Grape Seed Extract On Shear Bond Strength Of Resin Composite Bonded To Deciduous Dentin" (An In Vitro Study)

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***Abstract***
***Background:*** *The Dentin Is A Complicated, Mineralized, Three-Dimensional Tissue, Composed Of Several Tubules, That Extends From The Pulp To The Dentino-Enamel Junction, As Well As Intertubular And Peritubular Dentin. Its Weight Is Distributed As 70% Of Its Bulk In Minerals, While The Organic Component And The Fluids Occupy 20% And 10% Respectively.*
***Objective:*** *This Study Aimed To Evaluate The Effect Of Collagen Cross-Linker Proanthocyanidin (Pa) On: Shear Bond Strength.*
***Materials And Methods:*** *In This Experimental Study, 14 Teeth Were Intact, Carries Free Human Molar Teeth, And Teeth With Any Sign Of Crack Or Developmental Defect Were Excluded.*
***Results:*** *The Mean Of Shear Bond Strength In Total Etch Groups Increased From (8.96±2.23) In The Control Group (Tc) To (12.20±3.61) In The Grape Seeds Group (T-Gse), And This Increase Was Not Statistically Significant (P < 0.05). Whereas The Mean Of Shear Bond In Self-Etch Groups Increased From (6.65±2.82) In The Control Group (Sc) To (11.81±4.68) In The Grape Seeds Group (S-Gse), And This Increase Was Statistically Significant Depending On The Means We Can Arrange The Groups (Descending Order) As The Following T-Gse > S-Gse > Tc > Sc*
***Conclusion:*** *The Use Of 6.5% Ges, As Collagen Cross-Linkers, Significantly Increased The Resin-Dentin Shear Bond Strength When Compared To The Control.*
***Keywords:*** *Proanthocyanidins, Matrix Metalloproteinases*

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Date of Submission: 02-07-2023 Date of Acceptance: 12-07-2023

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## I. INTRODUCTION

The use of composite restorations has transfigured today's dental practice by being able to replace lost tooth tissue invisibly and conservatively with immense success [(1)]. Achieving an efficient and stable bond between composite and dentin remains a challenge in restorative dentistry [(2)].

While bonding to enamel is authentic over time, bonding to dentin is a great challenge. Dentin is a complex hydrated structure its properties vary with the preparation depth of the tooth [(3)]. Dentin consists of an organic phase of type one collagen and an inorganic phase saturated with hydroxyapatite crystals [(4)] Proper bonding to dentin depends on efficient infiltration of resin into the demineralized microporous collagen and formation of hybrid layer [(5)] and stable hybrid layer between resin and collagen microfibers assure a durable bond [(6)].

An important challenge to dentin bond durability is the degradation of collagen from the matrix-bound proteases, namely matrix metalloproteinases (MMPs) and cysteine cathepsins. Pretreatment of the bonding substrate with agents that inhibit the activity of MMPs might improve bond durability. Several chemicals, both natural and synthetic, which can increase collagen cross-links are used to improve bond durability. Proanthocyanidins (PAs) are oligomeric flavonoids found in high concentrations in grape seed, pine bark, cranberries, lemon tree bark, and hazelnut tree leaves [(7)].

few studies have been done to find the role of grape seed extract (GSE) in improving the bonding characteristics of dental adhesives, this study was designed to evaluate the efficacy of GSE on composite-dentin bond strength in deciduous teeth.

**AIM OF THE STUDY**

The aim of this study is to evaluate the effect of collagen cross linker Proanthocyanidin (PA) on: Shear bond strength,

## II. MATERIALS AND METHODS

### I- Materials:

**Table 1:** The materials used in this study

| Brand and manufacturer | Batch no. | Chemical Composition | |
|---|---|---|---|
| Grape seed extract Powder prescribed for life, China) Product Verified by CoA, USA. | SBT-S030164 | 99 % Seed, Proanthocynidins 115.22% Solvent Used: Ethyl-Alcohol |  |
| Single bond Universal (3M ESPE, Paul, MN 55144-1000 USA) | 7439597 | MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane. |  |
| Adper™ Single Bond 2,3M ESPE, ST. Paul, MN 55144-1000 USA) | NA93543 | bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, 10 vol% of 5-nm silica nanofiller, initiators, water, ethanol. Abbreviations: bis-GMA: bisphenol-A glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate |  |
| Filtek Z250 XT Nano Hybird Universal Restorative- (shade A2), / 3M EPSE, St Paul, MN, 55144-1000 USA | NC51086 | BIS-GMA, UDMA, BIS-EMA, TEGDMA. Silica particle 20 nm and Zirconia/Silica particle 10-0.1 microns (%67.8 by volume |  |

### II- Methods:

**1) Study Design**

This study is an in-vitro experimental study.

**2) Sample Size Estimation:**

According to the result of Paulose and Fawzy. 2017 [8], and by adopting an alpha (α) level of 0.05 (5%), beta (β) level of 0.20 (20%). i.e., power =95% and the predicted sample size (n) was found to be total of (28) samples i.e. (7) for each group.

**3) Study sample:**

Fourteen (n= 14), primary molars were collected from the outpatient clinic of pediatric Dentistry and Dental Public Health Department, faculty of dentistry, Ain Shams University.

**4) Sample selection:**

The teeth collected for this study were sound and showed no [8]:

- Cacks,
- Developmental defects,
- Dental fluorosis,
- Caries.

**5) Sample grouping**

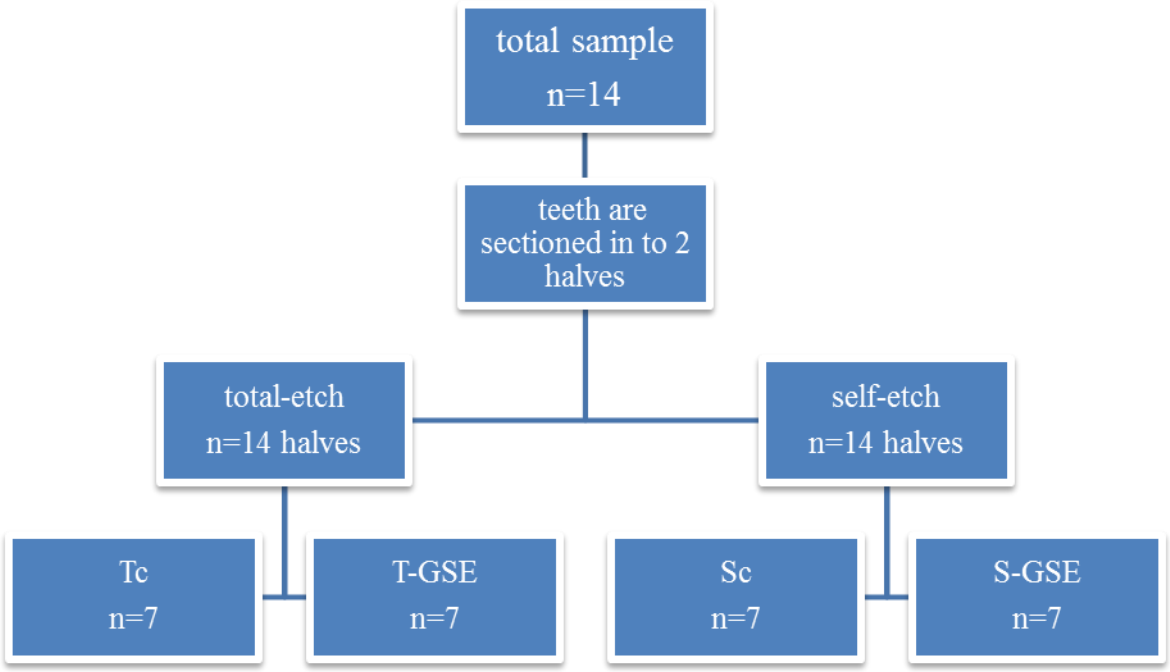


**Fig. 1:** Grouping of the study samples.
(Tc): total-etch control,
(T-GSE): total -etch with grape seeds extract
(Sc): self-etch control
(S-GSE): self-etch with grape seeds extract

**Procedure:**

**1- Preparation of Grape seed extract (GSE):**

6.5 grams of 95% grape seed extract powder (figure 2) was added to 100 ml of the solvent (acetone-water 30:70) pH was adjusted to 7 by adding 1m NaOH drop by drop [9].


**Fig. 2:** Grape seed powder


**Fig. 3:** 6.5% grape seed extract

**4- Shear bond strength test**

**a) Fabrication of the Mold:**

A specially constructed split Teflon mold (figure 4) with circular shaped central hole with a size of (3 mm x 2 mm) was fabricated for application of the resin composite on the tooth surface. An aluminum ring was fabricated to surround and hold the two halves of the split Teflon mold during packing of resin composite materials [(10)].

**b) Sample preparation for shear bond strength test:**

The selected teeth were split into buccal, lingual or palatal halves then were embedded into the acrylic resin mold (figure 5) with buccal, lingual or palatal surface facing upwards. The surfaces were grounded flat under copious amount of coolant water to remove enamel and expose dentin [(11,12)]. Then teeth were randomly divided according to the treatment and bonding system used (figure 6) [(12).]

- (Tc) group, after etching with 35% phosphoric acid for 15 sec. were rinsed for 10 seconds. The excess water removed with a cotton pellet; 2-3 SB coats were applied for 15 sec then they were air thinned gently for 5 sec to evaporate the solvent. Lastly, they were light cured for 10 seconds [(8)].
- (T-GSE) group, Grape seed extract (GSE) was applied for 10 minutes, the excess GSE was absorbed using a cotton pellet before the bonding agent Adper Single bond 2 (SB) was applied [(8,13)].
- (Sc) group was treated with Universal Bond (SU). The adhesive was applied and rubbed for 20 seconds. A gentle stream of air was directed over the liquid to ensure that the solvent was totally evaporated [(8)].
- (S-GSE) group was treated with GSE. GSE was applied for 10 minutes. The excess solution was absorbed with cotton pellet before the bonding agent SU was applied [(8,13)].

All groups received a cylindrical composite resin with a 3-mm diameter and 2-mm height using Teflon mold (figure 7). The specimens were immersed in distilled water for storage until they were subjected to testing procedure. All specimens were subjected to thermocycling procedure [(14)].

**a. Shear Bond Strength Test:**

All the specimens were subjected to shear bond strength test at the interface between composite cylinders and dentine using universal testing machine[*(b).] The load was applied until restoration failure occurred (figure 8), then the load of failure was measured in Newtons [(15).]

**b. Fracture Mode Analysis:**

After shear bond test was conducted, all specimens in the test groups were viewed using a USB digital-microscope and the images were captured and transferred to an IBM personal computer equipped with the Image-tool software (Image J 1.43U, National Institute of Health, USA) to determine the failure mode pattern (Figure 9) according to the following categorization [(13,16)].

- Cohesive failure: Fracture occurred within the resin composite or dentin
- Adhesive failure: Fracture occurred at the adhesive/dentin interface
- Mixed failure: Fracture occurred at the adhesive/dentin interface accompanied with part of the resin composite left on the dentin surface.

[(b)] INSTRON, model 3345, USA, the test was performed in a Mohamed Abbas privet lab in Nasr city.
[(c)] Digital-microscope (U500x Digital Microscope, Guangdong, China), magnification x35.

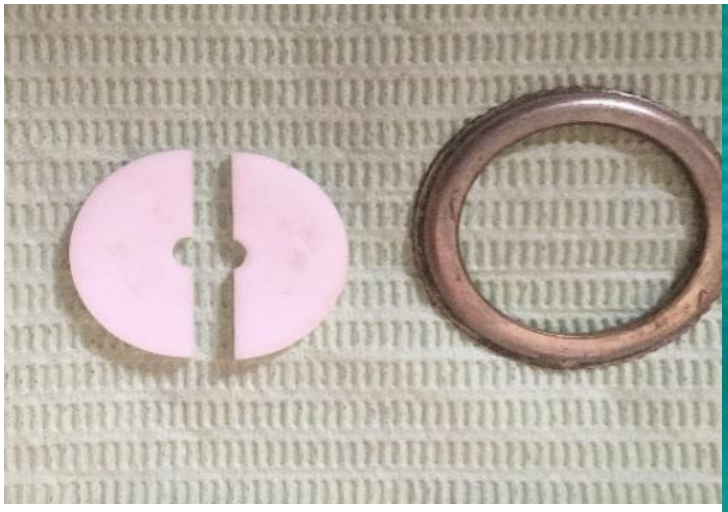

**Fig. 4:** Mold measuring 3×2 mm

**Fig. 5:** Tooth embedded into the acrylic resin mold

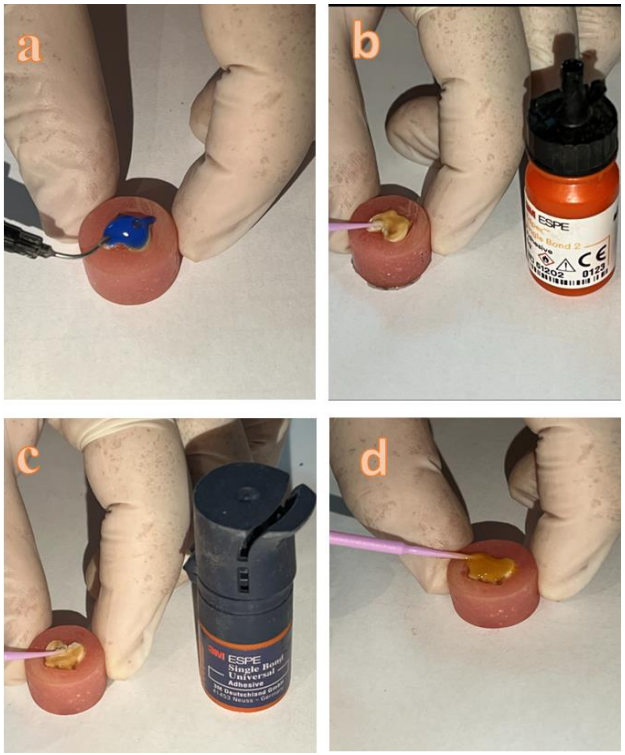


**Fig. 6: a-** Dentin surface etched with 35% phosphoric acid, **b-**Single Bond 2 applied to dentin surface, **c-** Universal bond (self-etch) mode applied to dentin surface, **d-**GSE applied to the bonded dentin

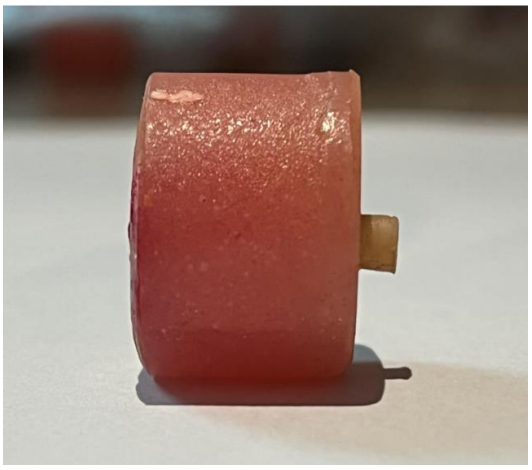

**Fig. 7:** "Side view "of cylindrical composite resin bonded dentin surface

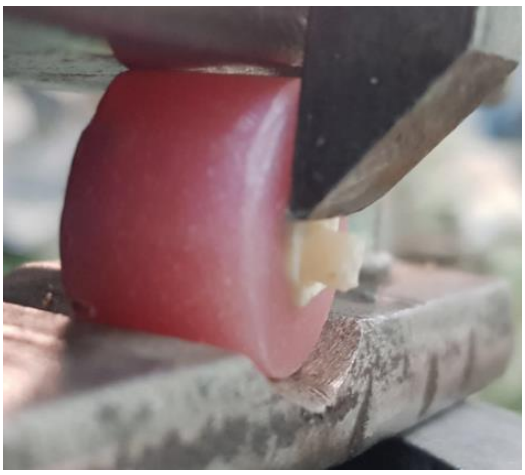

**Fig. 8:** Specimen subjected to load using chisel attachment

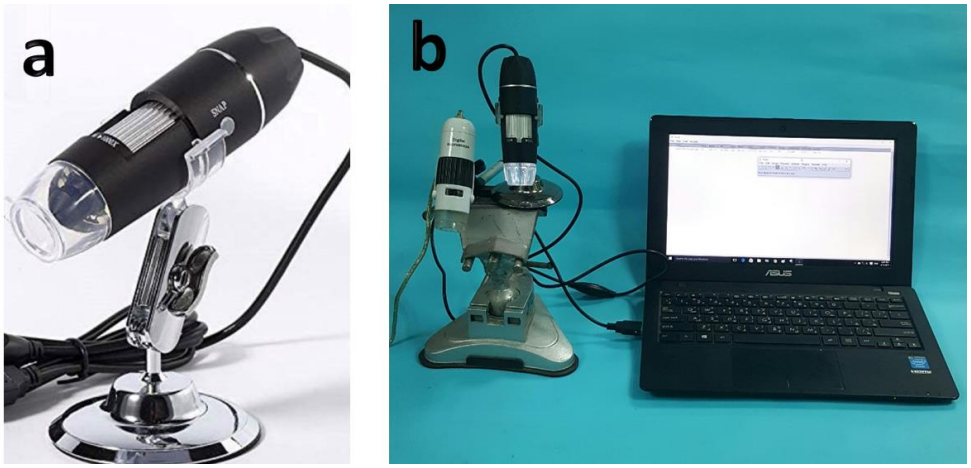


**Fig. 9:** a& b: Digital Microscope

**Statistical analysis**

The collected data were revised, coded, tabulated, and introduced to PC using a statistical package for social science (SPSS version 23,2015). IBM Co. USA. Statistical analysis of the results was performed by applying the ANOVA test (one way) followed by a post hoc test for multiple comparisons between different variables. Descriptive Statistics:

- Mean
- Standard deviation (±SD)

p- value level of significance:

- $P > 0.05$: non significance (NS)
- $P \leq 0.05$: significance (S)

## III. RESULTS

**I- Shear bond strength test:**

According to the results from (Table 2) The mean of shear bond strength in **Total etch groups** increased from (8.96±2.23) in the control group (Tc) to (12.20±3.61) in the grape seeds group (T-GSE), however, this increase was not statistically significant ($P > 0.05$),

Whereas the mean of shear bond in **Self-etch groups** increased from (6.65±2.82) in the control group (Sc) to (11.81±4.68) in the grape seeds group (S-GSE), and this increase was statistically significant ($P < 0.05$).

**Table 2:** Mean ±SD of Shear bond strength (MPa):

| Shear bond (MPa) | Control | GSE | P-value ** |
|---|---|---|---|
| Total etch | 8.96 ±2.23 | 12.20 ±3.61 | 0.319 $^{NS}$ |
| Self- Etch | 6.65±282 | 11.81±4.68 | 0.047 $^{S}$ |

P-value for intragroup comparison (Control vs. GSE), and it is conceded statistically significant if P value ≤ 0.05. NS: non-significant (p>0.05)

- ***Intergroup comparison***

The results of Post hoc test indicated there were no statistically significant differences in the mean of shear bond strength between Tc and

T-GSE (the means have the same superscript Letter (x), There was no statistically significant difference in the mean of shear bond strength between Tc and S-GSE (the means have the same superscript Letter (x).

Moreover, there was no statistically significant difference in the mean values of shear bond strength between Tc and Sc (the means have the same superscript Letter (y). And there was statistically significant difference in the mean of shear bond strength values between T-GSE and S-GSE (the means have the same superscript Letter (x) (table 3).

The overall P-value for inter-group comparison was statistically significant at the 0.05 level ($P < 0.05$), meaning there was a statistically significant difference between the subgroups in the mean of Shearing Bond Strength. Depending on the means we can arrange the groups (Descending order) as the following T-GSE > S-GSE > Tc > Sc

**Table 3:** Intergroup comparison of shear bond strength (MPa) for all groups

| **Groups** | **Tc** | **T-GSE** | **Sc** | **S-GSE** | **P-value**** |
|---|---|---|---|---|---|
| Shear bond strength (MPa) | $8.96\pm2.23^{xy}$ | $12.20\pm3.61^{x}$ | $6.65\pm2.82^{y}$ | 11.81±4.68x | **0.02**$^{S}$ |

- ** Overall p-value for Inter-group comparison (ANOVA results).
- S: statistically significant ($P \leq 0.05$.)
- Small letters for intergroup comparison (TC vs. T-GSE vs. SC vs. S-GSE) and the means with different superscripts (in the same row) are statistically significant different at $P \leq 0.05$.

**II- Failure mode Analysis:**

Failure mode analysis of the tested groups is presented as percentages and was classified as (A)Adhesive, (C)Cohesive and (M)Mixed. Mixed failures were predominant in all groups. (T-GSE) group showed a higher percentage of mixed failures compared to the other groups. (Table 4)

**Table 4:** Comparison of the failure mode distribution between all groups.

| | **Tc** | **T-GSE** | **Sc** | **S-GSE** |
|---|---|---|---|---|
| Adhesive failure(A) | 28.6% | 0% | 14.3% | 14.2% |
| Mixed failure(M) | 71.4% | 100% | 71.4% | 42.9% |
| Cohesive failure (C) | 0% | 0% | 14.3% | 42.9% |

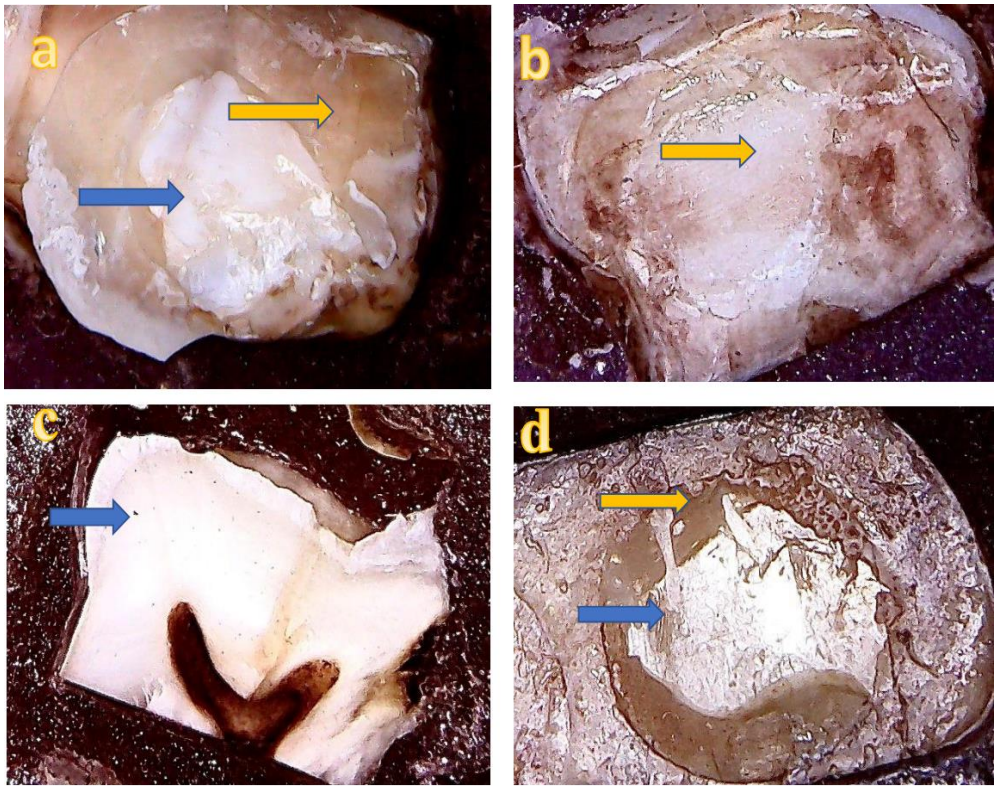

**Fig. 10:** Failure modes as seen under stereomicroscope. a) Adhesive failure, (b) Cohesive failure in bond, (C) cohesive failure in dentin, d) mixed failure. Blue arrows are pointing to dentin, yellow arrows are point in to tooth-restoration interface.

## IV. DISCUSSION

Dentin comprises the largest dental structure available for bonding. The ultimate goal of a bonded restoration is to attain an intimate adaptation of the restorative material with the dental substrate [16,18]. This task is difficult to be achieved as the bonding process is different for enamel and dentin. While enamel is predominantly mineral, dentin contains a significant amount of water and organic material, mainly type I collagen [15] .

The degradation of resin - dentin bond occurs mainly due to the deterioration of the dentin collagen and the polymerized resin. Collagen degradation could be attributed to the release of dentinal endogenous protease that leads to the destruction of the collagen matrix, compromising the bond strength. Hence, biomodification of dentin using collagen cross-linkers has been investigated as a method of improving the mechanical and biological characteristics of dentin [18] .

Natural cross-linkers were recently suggested as a safe and biocompatible approach to dentin biomodification rather than cytotoxic synthetic cross-linkers such as glutaraldehyde [19] .

Grape seed extract (GSE) is one of the most widely investigated natural cross-linkers as it is the most abundant in terms of proanthocyanidine content among other natural fruits and berries [10,11] . The used concentration (6.5%) is the most common and most effective concentration used in literature [12].

Grape seed extract main use in conservative dentistry as a cross-linking agent during bonding of restoratives and adhesives [10,11] . Macedo et.al., [12] reported that the application of grape seed extract to dentin significantly improved bond strength.

Many studies measured the effect of grape seed extract on dentin bond strength after 1 minute ,30 minutes, 1 hour, and 4 hours [12,20,21]. Although the longer treatment periods provided higher and more significant effect, but none of these time frames were clinically applicable. Treatment for 10 minutes was selected based on previous studies that attempted to improve the resin-dentin bond by clinically relevant 10 minutes treatment [8,12,18] .

The current study was formulated to evaluate the effect of effect of grape seed extract on nanoleakage, length of resin tags and shear bond strength. Based statistical analysis and previous studies, Total sample size of 28 deciduous molars was sufficient, deciduous mandibular second molars were selected due to their satisfactory mesio-distal width [8] .

Once the sample preparation was completed thermocycling which involved 500 cycles through water baths at 5$^{o}$C and 55$^{o}$ C was done [22] , to simulate one of the many challenges that occur in the oral environment [23] .

The two main strategies that are currently in use for adhesive bonding to enamel and dentin are the total-etch technique and the self-etch technique. Although etch-and-rinse technique is still considered as the most effective approach to achieve efficient and stable bonding to enamel and dentin, the imperfect penetration of the resin into the dentin is inevitable for total-etch adhesives and, as well, to a lesser extent, self-etching adhesive systems might lead to the hydrolytic degradation of the bonds [24-29]. The degradation of resin dentin bonds can be categorized into three major groups, including the hydrolytic degradation of the collagen matrix, the hydrolytic degradation of the bonding resin within the hybrid layer, and the hydrolytic degradation of the resin [30] .

Shear bond test was selected for this study as it is the most commonly used test for determining the bonding performance of adhesives as it is the fastest and simplest technique and does not need additional specimen preparation after the bonding procedures, also it does not need any special equipment to prepare or test the bonded specimen, however, the test is not free from any defects regarding the stress distribution at the surface [31] .

In the current study the mean of shear bond strength in the specimens treated with grape seed extract was higher than that of the control groups. This could be attributed to four different mechanisms that have been proposed for the interactions between collagen crosslinkers and proteins: covalent interaction, [32,33] ionic interaction, [34] hydrogen bonding interaction, [34] .

The present study was in harmony also with the findings of previous studies [8,21] which showed that the application of 6.5% grape seed extract for different period of time to demineralized dentin surface significantly improved the resin-dentin bond strength. In all these studies, it was reported that the treatment of dentin with natural cross-linking agents as grape seed extract produced a significant enhancement in the mechanical properties and structural stability of the dentin matrix and hence increased dentin bond strength.

On the contrary, Hass et.al.,[35] and El Gindy, et al., [36] evaluated the effect of natural cross-linkers on the resin-dentin bond strength and reported that the use of grape seed extract did not affect the bond strength,

this could be due to the shorter treatment time of dentin (1 minute) they adopted in their researches which could have been insufficient to improve the resin-dentin bond strength. This explanation was also supported by Castellan et. al., [(12)] who confirmed that the effect of natural dentin crosslinkers was dependent on the duration of the treatment.

Moreover, Mojtahedi, et al., [(37)] studied the effect of synthetic grape seed extract on the shear bond strength of composite resin to dentin and concluded that grape seed extract could not improve the bond strength between adhesive and dentin this may be due to the use of grape seed extract with high concentration which might not have penetrated the tissues efficiently, reducing the chance for providing cross-links in the collagen matrix.

Furthermore, after shear bond test, the fractured samples in each group were observed using stereomicroscope to determine the mode of failure which can provide highly valuable information for the detection of weaknesses in different testing methodologies so as to improve their reliability, providing results that represent the actual strength of adhesive junction [(20)].

As regards the evaluation of the fracture mode analysis in the present study mixed failures were predominant in all groups. Total etch group treated with grape seed extract showed a higher percentage of mixed failures compared to the rest this may indicate that the resin dentin bond was strengthened in that group. fracture modes were under a stereomicroscope [(20)].

The results of the fracture mode of analysis obtained in the current study was in line with shear bond strength as previous studies [(38-50)] revealed that a larger percentage of mixed fracture are associated with high bond strength.

Based on the results obtained in this study, it could be highlighted that grape seed extract was able to enhance the bond strength which appear to be promising natural cross-linker in term of dentin biomodifications. However further studies investigating the effect of grape seed extract on deciduous teeth are still required.

## V. CONCLUSION

The use of 6.5% GES, as collagen cross-linkers, significantly increased the resin-dentin shear bond strength when compared to the control.

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