



ORIGINAL ARTICLE

The Effect of Dentin Biomodification using Naturally Derived MMP Inhibitors on Microtensile Bond Strength of Composite Resin - An *In vitro* Study

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ABSTRACT

Aim of the study is to evaluate and compare the effect of grape seed extract (GSE) and amla extract (*Phyllanthus emblica*) as natural matrix metalloproteinases (MMP) inhibitors on microtensile bond strength (μ TBS) of composite resin to dentin. Thirty human mandibular premolars were assigned equally to three groups: GSE-treated, Amla extract-treated, and control (distilled water). Following 37% phosphoric acid etching, the respective treatments were applied for 60 s. Teeth were restored using a universal adhesive (Tetric NBond) and composite (Tetric NCeram). Specimens were sectioned into beams (1 × 1mm) and subjected to μ TBS testing at 0.5 mm/min. Statistical analysis included one-way ANOVA and Bonferroni post-hoc tests ($\alpha = 0.05$). The GSE group demonstrated significantly higher mean μ TBS (≈ 35 MPa) than both the Amla group (≈ 28 MPa) and control (≈ 20 MPa) ($p < 0.05$). Amla-treated specimens also showed a statistically significant improvement over control. Both grape seed and amla extracts enhance μ TBS compared to untreated dentin, with grape seed extract showing superior efficacy. These results support the potential clinical application of natural MMP inhibitors to improve bond durability.

Keywords: Dentin biomodification; Grape seed extract; Amla extract; Microtensile bond strength; MMP inhibitors

1 INTRODUCTION

The long-term clinical success of resin composite restorations largely depends on the durability of the bonds formed between adhesive systems and tooth structures, particularly enamel and dentin¹. Bonds created between resin and dentin using hydrophilic adhesives gradually deteriorate due to time-dependent hydrolytic and enzymatic processes^{2, 3}. Total-etch adhesives often fail to fully infiltrate acid-etched dentin, leaving denuded collagen fibrils at the bottom of the hybrid layer⁴. Collagen fibrils within the hybrid layer are progressively degraded by endogenous dentinal MMPs especially MMP-2 and MMP-9 resulting in reduced bond strength with aging. Human dentin contains several matrix metalloproteinases (MMPs), including MMP-2, MMP-8, MMP-9, and MMP-20, which belong to a class of zinc- and calcium-dependent endopeptidases. Following resin bonding, these MMPs can become gradually activated, leading to the degradation of collagen fibrils beneath the hybrid layer that were not fully infiltrated by adhesive resin⁵.

Numerous *in vitro* studies have explored the use of MMP inhibitors and collagen-stabilizing agents—such as riboflavin, green tea extract (epigallocatechin-3-gallate), chlorhexidine (CHX), and glutaraldehyde—to suppress MMP activity and preserve the hybrid layer^{4, 6-8}. While these natural agents function as non-specific MMP inhibitors and cross-linkers, specific synthetic compounds like galardin, batimastat and carbodiimide have shown greater efficacy in collagen cross-linking and MMP inhibition^{1, 9}.

Emblia officinalis (commonly known as Indian gooseberry or amla) is a potent natural source of vitamin C, essential minerals, amino acids, and a variety of phenolic compounds¹⁰. Amla juice exhibits high acidity (pH ≈ 2.85), which is comparable to the pH of certain self-etching dental primers. Additionally, several studies have demonstrated the efficacy of *Emblia officinalis* extract as a potent matrix metalloproteinase (MMP) inhibitor, particularly in soft tissue models^{11, 12}.

Vitis vinifera, commonly known as grape, is a rich source of proanthocyanidins—natural polyphenolic compounds known for

their antioxidant, anti-inflammatory, and collagen cross-linking properties¹³.

Although numerous agents have been evaluated in the literature for their potential as MMP inhibitors, evidence supporting the use of naturally derived compounds continues to grow. Therefore, the present study aimed to evaluate the effect of *Vitis vinifera* (grape seed extract) and *Emblica officinalis* (amla) as natural MMP inhibitors and assess their influence on the microtensile bond strength of composite resin to dentin. The null hypothesis was that there would be no statistically significant difference in the microtensile bond strength between the *Vitis vinifera* and *Emblica officinalis* treatment groups.

2 MATERIAL AND METHODS

2.1 Sample Selection

A total of 30 freshly extracted, intact human mandibular premolar teeth were collected from Department of Oral and Maxillofacial Surgery, KVG Dental College for this *in vitro* study. All teeth were thoroughly cleaned of surface debris using a rubber cup and pumice slurry with a slow speed micromotor handpiece. Following cleaning, the teeth were stored in distilled water containing 0.2% thymol (Thymol; Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 48 hours to ensure disinfection and hydration. Teeth exhibiting carious or non-carious lesions, restorations, fractures, crack lines, discoloration, fluorosis, enamel hypoplasia, or other developmental defects were excluded from the study.

2.2 Specimen Preparation

The roots of 30 selected human mandibular premolars were sectioned 2 mm apical to the cemento-enamel junction using a water-cooled diamond disc. The occlusal surfaces were then flattened to expose mid-coronal dentin, producing dentinal slabs with a standardized thickness of 3 mm. These surfaces were finished with 600-grit silicon carbide abrasive paper under running water to produce a uniform smear layer.

Each dentin slab was embedded in cold-cure acrylic resin (DPI-RR Cold Cure; Dental Products India, Mumbai, India) using square rubber molds measuring 25 × 25 mm to ensure standardization.

2.3 Group Allocation

The stored 30 samples were randomly divided into three groups of 10 per each group:

1. GSE group:(n-10) 100% grape seed extract.
2. Amla group:(n-10) 100% amla extract.
3. Control:(n-10) distilled water.

Acid etching was standardized across all groups. 37% orthophosphoric acid (OPA) was applied to the dentin surface for 15 seconds using microbrush applicator tips, followed by rinsing with distilled water for 10 seconds and gentle air-drying for 5 seconds. Subsequently, the respective matrix metalloproteinase (MMP) inhibitors were applied for 60 seconds, rinsed thoroughly, and air-dried.

2.4 Bonding Protocol

All specimens, regardless of group allocation, were treated with a universal dentin bonding agent (Tetric N-Bond, Ivoclar Vivadent). The adhesive was applied actively using a microbrush for 20 seconds, air-dried gently for 5 seconds, and light-cured for 10 seconds using an LED curing unit (Woodpecker Medical Instrument; Guilin, China).

A nanohybrid composite resin (Tetric N-Ceram, Ivoclar Vivadent) was then incrementally built up inside a polyethylene mold (2 mm internal diameter), placed directly over the bonded dentin. Two horizontal increments of 1 mm each were placed and light-cured for 20 seconds per increment using a light source with an output of 400 mW/cm² at zero distance. After polymerization, the polyethylene tubes were carefully removed using a sharp blade. All specimens were stored in distilled water at 37°C for 7 days prior to testing.

2.5 Microtensile Bond Strength Testing

Specimen Preparation & Bond Testing: After storage in distilled water, each acrylic resin block with its bonded composite cylinders was secured to the lower compartment of a universal testing machine using tightening screws. The specimens were subjected to microtensile bond strength testing using a universal testing machine with a 5 kN load cell at a crosshead speed of 0.5 mm/min. The load was applied until failure occurred, and the data were recorded

2.6 Statistical Analysis

For statistical analysis, the average microtensile bond strength (mTBS) values were calculated to obtain the mean value of the bonding strength of each tooth. The mean microtensile bond strength values and standard deviations were determined for all groups. The normality of the data was checked using the Shapiro-Wilk test. One-way analysis of variance was used as a parametric test, followed by the post hoc Bonferroni test. A p value of more than 0.05 indicated no statistical significance.

3 RESULTS

Grape Seed Extract exhibited the highest mean microtensile bond strength at 17.30 MPa, whereas Amla Extract showed a moderate bond strength with a mean value of 13.30 MPa and Distilled Water recorded the lowest bond strength at a mean of 9.10 MPa. The p-value was < 0.001, indicating a highly significant difference in microtensile bond strength among the groups.

Table 1: Descriptive values of Micro tensile bond strength of Composite among studied groups. (MPa)

Groups	N	Mean (MPa)	Std. Deviation
DISTILLED WATER (Group-3)	10	9.10	.568
AMLA EXTRACT (Group-2)	10	13.30	.675
GRAPE SEED EXTRACT (Group-1)	10	17.30	.675

p-value < 0.005

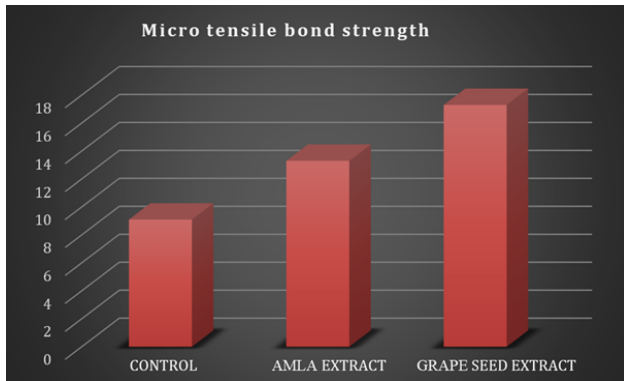


Fig. 1: Bar graph showing Micro tensile bond strength of Composite among studied groups. (MPa)

Table 2: Intergroup comparison of Micro tensile bond strength of Composite among studied groups. (MPa)

Sample (I)	Sample (II)	Mean Difference (I-II)	p-value
Amla Extract	Grape Seed Extract	-4.000*	.000*
	Control	4.200*	.000*
Grape Seed Extract	Amla Extract	4.000*	.000*
	Control	8.200*	.000*
Control	Amla Extract	-4.200*	.000*
	Grape Seed Extract	-8.200*	.000*

ANOVA revealed statistically significant differences among groups ($p < 0.05$). Bonferroni posthoc analysis confirmed GSE > Amla > Control (all pairwise; $p < 0.05$)

4 DISCUSSION

One of the key factors contributing to the deterioration of the hybrid layer is the hydrolytic and enzymatic breakdown of inadequately resin-infiltrated collagen fibrils by host-derived matrix metalloproteinases (MMPs)^{2, 5, 13}. The application of either natural or synthetic MMP inhibitors on dentin surfaces has been shown to reduce this degradation and enhance the durability of the resin-dentin bond^{4, 6-9, 14}. Amla extract, a known MMP inhibitor in soft tissue models^{11, 12}, also exhibits a highly acidic nature with a pH of 2.85¹⁰, comparable to that of self-etching primers. Therefore, the present study aimed to evaluate the effect of *Emblia officinalis* (amla) and *Vitis vinifera* (grape seed extract) as MMP inhibitors on dentinal substrates.

A study conducted in 2008 demonstrated that *Emblia officinalis* (amla) extract exhibits significant matrix metalloproteinase (MMP) inhibitory activity on human skin fibroblasts in a dose-dependent manner¹¹. Similar to *Emblia officinalis* (amla), various natural extracts such as *Camellia sinensis*, chitosan, and marine sponges have demonstrated selective inhibitory effects on MMP-9 activity¹⁴. The MMP-inhibitory potential of amla is likely attributed to its rich content of polyphenols and multiple hydroxyl groups.

Pretreatment with grape seed and amla extracts significantly improved composite resin bond strength to dentin, consistent with earlier studies demonstrating MMP inhibition and collagen stabilization by plant polyphenols. GSE's proanthocyanidins are particularly effective collagen cross-linkers, likely accounting for the superior bond strength. Amla polyphenols, though effective, may have lower cross-linking potency, explaining comparatively lower μ TBS enhancement.

From a clinical perspective, the use of GSE and amla as dentin pretreatments represents a promising biomimetic strategy for enhancing the longevity of adhesive restorations, especially in minimally invasive restorative approaches.

Limitations of this study include its *in vitro* design, limited sample size, and absence of long-term aging or nanoleakage data. However, further studies on varying concentrations, aging effects, clinical performance over time and clinical trials are necessary to validate their effectiveness and safety in real-world applications.

5 CONCLUSION

Within the limitations of this *in vitro* study, it can be concluded that grape seed extract significantly enhances the microtensile bond strength of composite resin to dentin when used as a pretreatment agent, outperforming both amla extract and the control (distilled water). The superior performance of grape seed extract can be attributed to its potent collagen cross-linking and MMP-inhibitory effects, resulting in more stable and durable hybrid layer formation. Amla extract also improved bond strength compared to the control, indicating its potential as a natural dentin biomodifier, though to a lesser extent than grape seed extract. These findings support the use of natural MMP inhibitors as effective, biocompatible alternatives to synthetic agents for improving the longevity of adhesive restorations. Further long-term and clinical studies are warranted to validate their effectiveness in real-world dental applications.

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