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Influence of two natural cross-linkers on microtensile bond strength durability – An in vitro study

Influência de dois reticuladores naturais na durabilidade da resistência de união à microtração – Um estudo in vitro

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ABSTRACT

Objective: to investigate the effect of two natural cross-linkers on microtensile bond strength (μTBS) and evaluate their influence on the durability of the resin dentin bonds. **Material and Methods:** the *Moringa oleifera* and *Centella asiatica* plant extracts were qualitatively tested with high-performance thin layer chromatography (HPTLC) for the presence of phenols. The phenolic content ranged from 27 to 30 gallic acid equivalents (GAE), μg/mg of dry weight. After etching, two concentrations (5% and 1%) of these two extracts were prepared and used as pretreatment liners on dentin. They were applied for a min. After restoration with resin composite, dentin resin beams were prepared. The study groups were 5% *Moringa*, 1% *Moringa* 5% *Centella* 1% *Centella*, and control (without cross-linker application). For each group, half of the samples underwent μTBS testing after 24 hours, while the remaining half were immersed in artificial saliva to assess the bond's longevity after 6 months of ageing. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. **Results:** both 5% and 1% *Moringa* showed a significant difference ($p<0.05$) compared to the other groups at both intervals. However, after ageing, the specimens in the control and 1% *Centella* groups resulted in a significant decrease in μTBS. **Conclusion:** overall, both concentrations of *Moringa* (5% and 1%) were effective in stabilising the bond during both intervals.

KEYWORDS

Collagenase; Endopeptidases; Matrix metalloproteinase; Moringa; Phenols.

RESUMO

Objetivo: investigar o efeito de dois reticuladores naturais na resistência de união (μTBS) à microtração e avaliar sua influência na durabilidade da adesão da resina à dentina. **Material e Métodos:** extratos das plantas Moringa oleifera e Centella asiatica foram qualitativamente testados através de cromatografia em camada fina de alta performance (HPTLC) para a presença de fenóis. O conteúdo fenólico alcançou entre 27 a 30 equivalentes de ácido gálico (GAE), μg/mg de peso seco. Após o condicionamento, duas concentrações (5% e 1%) dos extratos foram preparadas e utilizadas como forros de pré-tratamento em dentina. Eles foram aplicados por um minuto. Após a restauração com resina composta, palitos de dentina e resina foram preparados. Os grupos foram 5% Moringa, 1% Moringa, 5% Centella, 1% Centella e controle (sem aplicação de reticulador). Para cada grupo, metade das amostras foram submetidas ao teste μTBS após 24 horas, enquanto a outra metade foi imersa em saliva artificial para avaliar a longevidade adesiva após 6 meses de envelhecimento. Foi realizada análise estatística através de ANOVA 1-fator, seguido do teste post hoc de Tukey. **Resultados:** ambas as concentrações de 5% e 1% de Moringa demonstraram diferença significativa ($p<0.05$) comparadas aos outros grupos em ambos os intervalos. No entanto, após o envelhecimento, os espécimes dos geupos controle e 1% de Centella resultaram em uma redução significativa de μTBS. **Conclusão:** no geral, ambas as concentrações de Moringa (5% e 1%) foram efetivas em estabelecer a adesão em ambos os intervalos.

PALAVRAS-CHAVE

Colagenase; Endopeptidases; Matriz de metaloproteinase; Moringa; Fenóis.

INTRODUCTION

The field of esthetic dentistry has undergone a remarkable transformation with the advancements in adhesive materials, particularly the resin based composite restoration. These innovations have not only made these restorations more user friendly but have also contributed to soaring popularity in recent years [1,2]. The continuous development of adhesive materials has brought about a paradigm shift, thus facilitating reliable bonding of resin composite to both enamel and dentin, resulting in esthetically pleasing and durable restorations [3]. Moreover, the advent of minimally invasive operating methods has further enhanced the clinical performance and longevity of these restorations, ensuring optimal oral health outcomes [4].

Contemporary adhesive technology has made significant strides in achieving immediate bonding, regardless of the approach used. The durability of the adhesive bond in restorative dentistry is crucial for the restoration's longevity and clinical performance. However, challenges persist in maintaining the durability and the bond strength of these adhesives gradually decreases [5,6]. The causes identified for the degradation of the bond between resin and dentin are water sorption, hydrolysis of the hydrophilic resin component and activation of endogenous dentin matrix metalloproteinases (MMPs) [7].

MMPs are zinc- and calcium-dependent, host-derived collagenolytic endopeptidases that are primarily activated through proteolytic cleavage of their proenzyme (Zymogen) forms, converting them into active enzymes. The activation of MMPs can occur by enzymatic activation or by an acidic pH. The acidic environment occurs during pathological conditions such as caries or during restorative procedures [8]. The acidic pH can cause confirmational changes in the proenzyme structure, leading to the exposure of the active site and subsequent activation of MMPs [9].

The hybrid layer, a critical transition zone between the resin in the bonding agent and collagen in dentin, distributes the stresses generated during functioning and prevents the bond from breaking down over time [10]. The stability and integrity of collagen fibrils form the structural foundation of the hybrid layer and are essential for the durability of the resin-dentin bond. The collagen fibers with incomplete infiltration of resin below the hybrid layer are referred to as exposed collagen, which has several implications for the stability and longevity of the bond [11]. These exposed collagen fibers may put the hybrid layer at risk due to exposure to MMPs, which trigger the collagenolytic activity and weaken the bond strength [12]. Armstrong provided the first transmission electron micrographs of the deteriorating hybrid layer, demonstrating the loss of insoluble collagen fibrils [13]. The inhibition of these proteases or improving the collagen structure resistance to these enzymes is the primary approach for preventing the degradation of collagen fibers in hybrid layers [14].

To address this concern, the use of cross linking agents has gained attention as a potential strategy to enhance bond strength and improve durability of adhesive surfaces. The inherent collagen cross-linking contributes to the stability, strength, and function of the dentin matrix [15]. The application of exogenous collagen cross-linkers (Dentin biomodification) induces additional inter and intramolecular cross-links of collagen that aid not only in strengthening the dentin matrix but also decreasing the biodegradation rate [16,17]. Several synthetic and natural collagen cross-linkers have been extensively studied to improve the mechanical properties of demineralized dentin [18-20]. Thus, the application of external cross-linkers is an attempt to stabilize the exposed collagen below the hybrid layer to benefit the durability of the bond.

Glutaraldehyde is a well-known synthetic cross-linking agent for intermolecular covalent cross-linking, although concerns regarding cytotoxicity have restricted its use [21]. Natural plant extracts rich in potential polyphenol molecules promote cross-linking between the fibrils [15] and stabilize collagen [22]. They form effective hydrogen bonds between collagen polypeptides, making them less susceptible to collagenase attack and inhibiting MMPs with their antioxidant property [23]. Natural cross-linkers have always been a better choice over synthetic agents as they also demonstrate MMP inhibition with a high level of biocompatibility [22].

Adhesion to tooth structure should be simple to achieve, provide marginal seal, have clinical durability, and retentive strength. Microtensile bond strength test (immediate and aging) is the most widely accepted method to measure retention of dental resin composite [24].

The reduced cross-sectional area of specimens in μTBS allows faster water diffusion through the hybrid layer, thus mimicking the in vivo conditions [25].

The plant extracts of *Moringa* and *Centella* are rich in phenols and flavonoids. In our earlier study, the effect of 5% *Moringa*, 1% *Moringa,* 5% *Centella and* 1% *Centella* in reducing the collagen degradation and loss of dry mass was observed. In the pretreated groups, there was a decrease in collagen degradation, as evidenced by the reduced release of ICTP and less loss of dry mass. This stands in contrast to the untreated group. The efficiency of these cross-linkers was dose-dependent i.e, higher concentrations proved better at inhibiting MMPs, thus reducing collagen degradation. (unpublished).

The present manuscript serves as a continuation of that research building upon the preliminary findings and expanding the investigation to assess the effect of *Moringa* and *Centella* on the bond strength to dentin to characterize its clinical performance. While the detailed results and analysis of the previous study are not included in this manuscript, we have incorporated the relevant findings into our current research design and interpretation. Therefore, this study aimed to evaluate the μTBS of the resin composite to the dentin, pretreated with the same concentrations of *Moringa* and *Centella* (5% and 1%). The study aimed to test three hypotheses each focusing on different aspects of the influence of natural cross linkers on the bond strength of dentin resin interface. The first hypothesis proposed was (a) the natural cross-linkers, *Moringa* and *Centella* are rich in polyphenol content. The second hypothesis (b) that the pretreatment of demineralized dentin with *Moringa* and *Centella* before application of dentin-bonding agent would preserve the bond strength of the dentin–resin interface even after aging. The third hypothesis (c) aimed to investigate that there is no difference in bond strength with different concentrations of cross linkers. Hypothesis “a” and “b” were alternate hypotheses, while hypothesis “c” served as the null hypothesis.

MATERIAL AND METHODS

Plant collection and preparation of plant extract

The fresh leaves of *Moringa oleifera* and *Centella asiatica* were destalked, shade dried, and powdered using an electric blender. The shade-dried powder was subjected to hydroalcoholic extraction by cold percolation method. About 10g of the shade-dried and coarsely powdered plant material was soaked in 100 ml of 70% ethanol solution for 24h. After 24h, the extract was filtered using Whatman No.1 filter paper (Cytiva, United states). The filter cake was collected and dried at 37°C. The dried extract was stored at 4°C and used for HPTLC analysis. The extracts were prepared to the required concentration using dimethyl sulfoxide (DMSO).

High-performance thin layer chromatography [26]

High-performance thin layer liquid chromatography (HPTLC) was performed on a 10x10 cm preactivated silica gel 60F 254 plate. The extracts of the *Moringa oleifera* and *Centella asiatica* and standard gallic acid of the required concentration were prepared and spotted using CAMAG Linomat 5 applicator (Switzerland). They were allowed to develop in a twin trough chamber of size 10x10 cm at 25±5°C which was kept saturated with the solvent system Chloroform: Ethyl Acetate: Formic Acid at a ratio of 2.5:2.0.:0.8 in an ascending mode manner under room temperature. The solvent system was found suitable for gallic acid, which was selected by trial and error and confirmed through its specific Rf values. After development, a pencil was used to mark the solvent front, and a hair dryer was used to dry the plates. The densitometric scanning of the developed plates was done at 280nm using scanner 3 and photo-documented using CAMAG reprostar 3 (Switzerland) at 254nm and 366nm. The quantification of gallic acid in *Moringa oleifera* and *Centella asiatica* was confirmed, and the results were expressed as mg of gallic acid per milligram of extract.

Specimen preparation

Fifteen sound non-carious human third molar teeth, extracted from people aged 18 to 45 years, were collected from the Department of Oral Surgery Narayana Dental College, following approval from the Institutional Ethical Committee (IEC/NDCH/2021/SEP/P-24). The teeth were cleaned, and stored in distilled water containing 0.5% Chloramine T solution. The teeth were used within a month of extraction.

The teeth were embedded in rectangular molds of self-cure acrylic resin. The occlusal enamel was removed and a flat dentin surface was exposed under water cooling using a slow diamond cutter (model: Struers- Minitom, United States).

An 8-fluted carbide bur produced a uniform smear layer on the exposed dentin surfaces. The teeth were randomly allocated to 4 test groups (n=3) and one control group. The 4 test groups were 5% *Moringa oleifera*, 5% *Centella asiatica*, 1% *Moringa oleifera* and 1% *Centella asiatica*. All the surfaces were etched with 37% phosphoric acid for 15 seconds and rinsed thoroughly. To prepare the dentin surfaces, the test solutions were applied as a pretreatment liner using an applicator brush for one minute. Any excess solution was blotted and the surface was left to dry. The universal bonding agent (Ivoclar Tetric N-Bond Universal) was applied and rubbed for 15 seconds with a micro brush. After a dwelling period of 2min, it was air dried with a gentle air blow. There was no pretreatment liner application in the sample teeth that belonged to control group, and the bonding procedure was followed after etching. After applying the bonding agent, all the samples were cured for 20 seconds (Ivoclar Bluephase N polywave LED curing light). Around 4 mm of resin composite (Ivoclar Tetric N Ceram) was incrementally built and cured on entire dentin surfaces.

The samples were stored in distilled water for 24 hours and later subjected to longitudinal sectioning with a slow diamond (model: Struers-Minitom, United States) under copious irrigation. Longitudinal beams of dentin- resin composite of around 1 x 1mm were prepared. Around 8 to 12 beams were obtained per tooth. The beams from one group were randomly divided into two sub test groups. Each sub group had ten (n=10) samples. One sub group was tested immediately for bond strength, while the other sub group was stored in artificial saliva for six months to evaluate the impact of aging on bond strength. Thus, a total of 50 samples were tested immediately and another 50 after six months. The storage media (artificial saliva) was replaced once in 15 days.

Microtensile bond strength (μTBS) testing

The bond strength testing was performed after one day and six months of storage. The exact dimension of each beam was measured with a digital caliper to determine the bond area accurately. The specimens were fixed, one at a time, to a customized jig (Geraldeli's Jig) using cyanoacrylate glue, ensuring that the micro-stick was parallel to the direction of the test block and mounted on a Universal Testing Machine (Instron E300). A tensile load was applied until failure at a crosshead speed of 1 mm/min. The μTBS in MPa at fracture was calculated by dividing the load to fracture by the surface area of the cross-section of the micro-sticks (bond area in mm^2) according to the formula MPa = N/ (a x b) where a and b are the dimensions of the beam at resin dentin interface. (Width and thickness).

Fracture mode analysis

The fractured beams were observed under a stereomicroscope (Lawrence Mayo Model. NO: LM-52-3621) at 80X magnification with transmitted LED light to determine whether the mode of failure was an adhesive failure (A), Mixed (M), cohesive failure in dentin (CD) or a cohesive failure in resin composite (CC).

SEM analysis

The fractured beams were cleaned with ethanol to remove any loose debris. The sample was later mounted on the brass stubs using carbon tape. Then the samples were placed in a vacuum chamber for sputtering and were platinum coated for 40 seconds. The images were captured in a set of magnifications to study the fracture site (FE-SEM IT800, JEOL).

Statistical analysis

The obtained data were statistically analyzed using unpaired t-test to examine if there was a difference in μTBS between the samples before and after the storage period (after 24 hours and 6 months). One-way ANOVA test was used to determine the effects of the cross linker and storage time on μTBS among different groups. The multiple pairwise comparisons among group means were made using Post hoc Tukey's test. Statistical significance was set at $p = 0.05$. All data analysis was performed by SPSS 20.0 software package (SPSS, Chicago, IL, USA).

RESULTS

Assessment of phenols

Based on the results obtained from HPTLC analysis, it was found that the Rf value of standard gallic acid and the Rf value of a peak in the plant extract was identical, confirming the presence of gallic acid in the plant extract.

The standard curve of gallic acid was considered and the quantification was estimated as gallic acid equivalents (GAE), μg/mg of dry weight. All determinations were performed in triplicate (n = 3). The amount of gallic acid in the *Moringa* was 29.93 μg of GA/mg of extract, and in *Centella* extract, it was found to be 27.97 micrograms per milligram of plant extract. Figure 1 and Figure 2 represent the photo document 254nm and 366nm and the Peak area of hydroalcoholic extract of *Moringa* (10μl) and *Centella* (10μl), respectively.

μTBS

The mean μTBS of all groups at different time intervals are given in Table I. μTBS values at 1 day and 6 months for each group were compared using unpaired t-test, and it did not show any significant difference except for Group 4 (1% *Centella*).

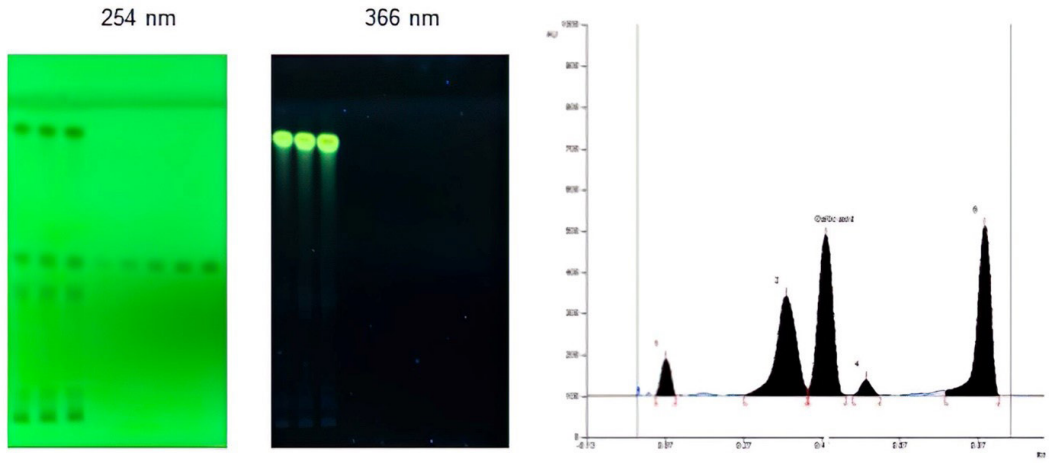

Figure 1 - Chromatograms obtained from separation of plant extracts and peak areas of hydroalcoholic extract of *Moringa Oleifera* in HPTLC analysis.

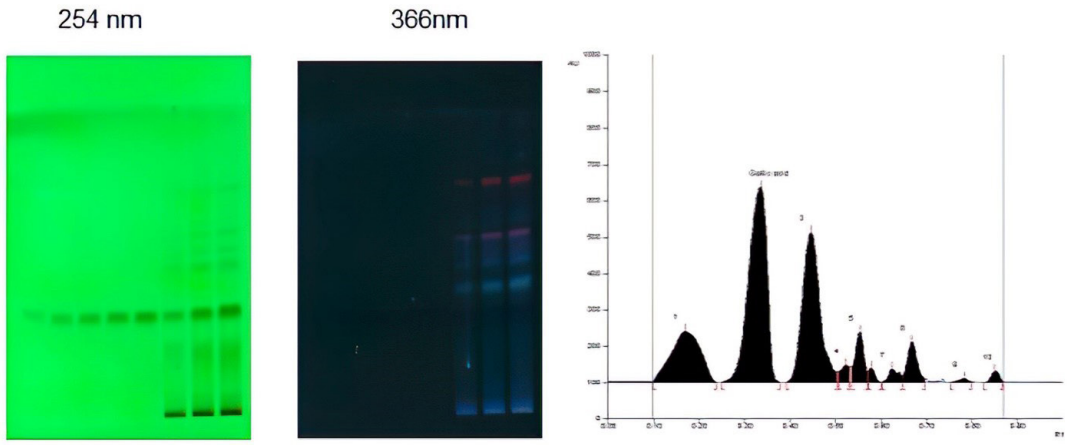

Figure 2 - Chromatogram and peak areas of hydroalcoholic extract of *Centella asiatica* in HPTLC analysis.

Table I - Comparison of mean μTBS values of all tested groups at two-time intervals

GROUPS	1 DAY	6 MONTHS	UNPAIRED T-TEST	Bond strength % change (related to time)
5% MORINGA	23.45±5.89	28.60±7.88	0.402(NS)	22.3% ∧
5% CENTELLA	14.28±4.04	16.23±4.80	0.340(NS)	14.3% ∧
1% MORINGA	18.62±5.31	18.79±5.40	0.944(NS)	0.912% ∧
1% CENTELLA	14.81±3.75	10.09±4.80	**0.025***	- 31.8% ∨
CONTROL	14.43±5.26	10.57±4.10	0.084(NS)	- 26.4% ∨
ONE WAY ANOVA	**<0.001***	**<0.001***		

∧ indicates increase in bond strength %. ∨ decrease in bond strength %. p<0.05* significant. (NS) = non-significant.

Figure 3 shows the difference in mean μTBS values of all groups at day 1 and 6 months. One-way ANOVA analysis of mean μTBS values in all the groups showed a significant difference ($p=0.001$) at 1-day and 6-month intervals. Group 1 (5% *Moringa*) showed the highest mean values of bond strength at both intervals. Group 4 (1% *Centella*) and 5 (Control) showed the least values. 5% *Moringa* showed an appreciable increase in bond strength at the 6-month interval (22.3%), whereas 1% *Centella* showed a decrease in bond strength (31.8%) similar to control (26.4%).

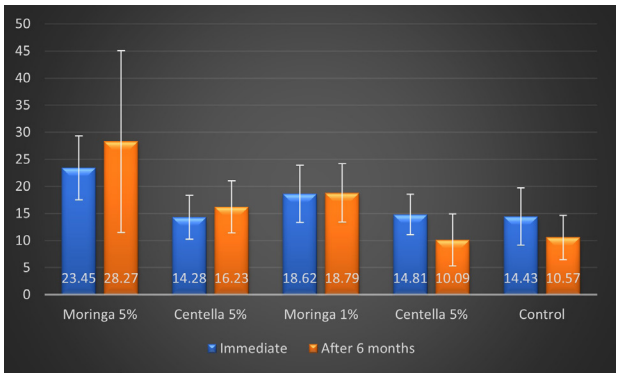

Figure 3 - Mean μTBS values of all groups at 1-day and 6 months intervals.

After conducting an ANOVA, a significant difference was found in the group comparison. To further investigate this, the Post hoc Tukey test was utilized (refer to Table II). At the 1-day interval, 5% *Moringa* showed a significant difference compared to *Centella* 5%, 1%, and control group. At 6 months interval, there was a considerable difference between 5% *Moringa* with all the other groups. *1%* Moringa too showed a significant difference when compared to *1%* Centella and control group (at 6 month interval).

Stereo-microscopic analysis:

The failure mode of fractured beams was classified as Mixed, Adhesive or Cohesive (in Dentin or Resin Composite). Table III represents the observations made and analysis with Fisher's exact test. There was no significant difference in failure modes among all groups when compared at 1-day and 6-month intervals. 5% *Moringa* showed 70% adhesive failure when compared to control with 100% adhesive failure at 6-month intervals. But this finding was not statistically significant.

Table II - Post hoc Tukey – to test significance in between groups ($p<0.05$* significant)

INTERVALS	GROUPS		pVALUE
1 day	5% MORINGA	5% CENTELLA	0.001* [a]
		1% MORINGA	0.201(NS)
		1% CENTELLA	0.003* [c]
		CONTROL	0.002* [d]
	5%CENTELLA	1% MORINGA	0.297(NS)
		1% CENTELLA	0.999(NS)
		CONTROL	1.000(NS)
	1% MORINGA	1% CENTELLA	0.428(NS)
		CONTROL	0.331(NS)
	1%CENTELLA	CONTROL	1.000(NS)
After 6 months	5% MORINGA	5% CENTELLA	<0.001* [a]
		1% MORINGA	0.002* [b]
		1% CENTELLA	<0.001* [c]
		CONTROL	<0.001* [d]
	5% CENTELLA	1% MORINGA	0.84(NS)
		1% CENTELLA	0.116(NS)
		CONTROL	0.172(NS)
	1% MORINGA	1% CENTELLA	0.009* [e]
		CONTROL	0.015* [f]
	1%CENTELLA	CONTROL	1.000(NS)

[a,c,d] representing the statistical significance between the groups at both intervals. Where as [b,e,f] represents the significance at 6 months interval only. (NS) = no significance.

SEM analysis

The SEM image of the fracture site revealed distinct features indicative of the nature of the bond failure. Figure 4A-D represents the SEM images of beams that had adhesive failure and cohesive failures. In a cohesive failure, both the resin composite and dentin fracture surfaces show similar characteristics, indicating that the failure occurred within the material itself (Figure 4C). In contrast, adhesive failure results in a distinct separation between the resin composite and dentin, indicating a failure at the interface. The tubules within the dentin are visible and appear to be filled or plugged (Figure 4A), suggesting an attempt to bond the resin composite and dentin.

Table III - Fisher's exact test analysis of the mode of failure of dental beams in all test groups at both intervals. (within groups and in between groups)

GROUPS	1 DAY		6 MONTHS		P VALUE
	ADHESIVE FAILURE	COHESIVE FAILURE IN DENTIN /RESIN	ADHESIVE FAILURE	COHESIVE FAILURE IN DENTIN /RESIN	
5% MORINGA	9(90)	1(10)	7(70)	3(30)	0.264(NS)
5% CENTELLA	9(90)	1(10)	8(80)	2(20)	0.531(NS)
1% MORINGA	9(90)	1(10)	9(90)	1(10)	1.000(NS)
1% CENTELLA	9(90)	1(10)	8(80)	2(20)	0.531(NS)
CONTROL	8(80)	2(20)	10(100)	0(0)	0.136(NS)
FISHER'S EXACT TEST (p value)	0.944(NS)		0.424(NS)		

(NS) = no significance.

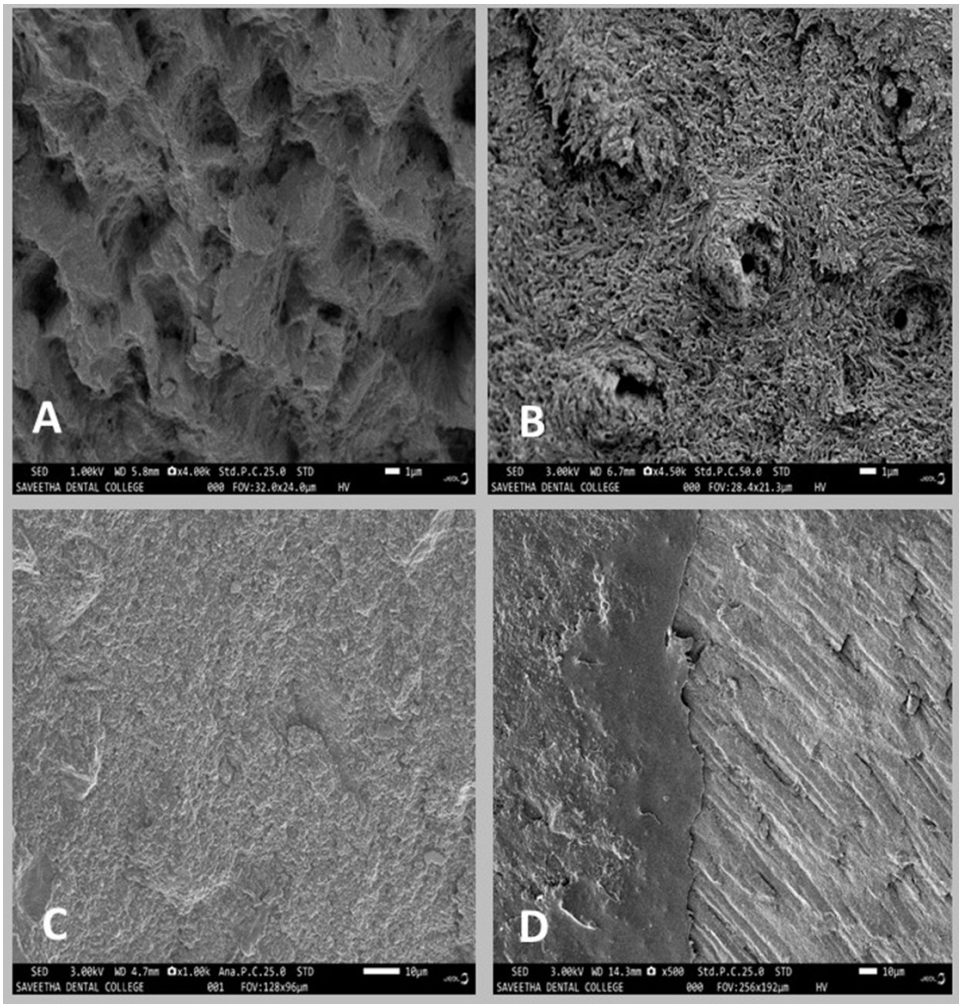

Figure 4 - SEM images: (A) representing adhesive failure- tubules with plugged dentin; (B) adhesive failure - tubules surrounded by exposed collagen; (C) cohesive failure in composite; and (D) cohesive failure (intact hybrid layer with resin composite).

DISCUSSION

The results of the present study partially supported hypothesis regarding the natural cross-linkers, *Moringa* and *Centella*, being rich in polyphenols (hypothesis 'a'). However, the effectiveness of preserving bond strength through the pretreatment of demineralized dentin with these extracts varied depending on the concentration used, leading to the rejection of hypothesis 'b' and 'c'.

Upon testing after a day, the mean bond strength values were similar for most groups except for 5% *Moringa*, which showed an appreciable increase in bond strength. Additionally, 1% *Moringa* showed slightly higher values than the control group. Notably *Moringa* demonstrated the ability to stabilise the bond strength at low concentrations while increasing it at higher concentrations. This suggests that *Moringa,* as a potent cross linker, preserves collagen fibre integrity and inhibits MMPs. On the other hand 5% *Centella*, maintained stable bond strength after ageing in contrast to the lower concentration of 1%. However, no significant increase in bond strength was observed in 5% *Centella* and 1% *Moringa* (as depicted in Figure 3). This indicates that while *Centella* may possess cross linking properties, it may not function as an effective MMP inhibitor like *Moringa*. It can be hypothesized that lack of cross linking agent in the control group, may have increased the likelihood of bond degradation and weakening over time.

Our previous study examining telopeptide ICTP release showed similar findings. Among the tested agents, 5% *Moringa* exhibited significant efficiency in inhibiting MMPs and cross linking the collagen, as evidenced by reduced telopeptide release from the dentin beams. Therefore, future research could focus on investigating the molecular nature of these two natural extracts. Although both extracts contained substantial phenolic content, the difference in their ability to preserve bond strength could be attributed to variations in molecular structure, complexity of the molecule, and penetration. A similar finding was reported by Aguiar et al. [16], where each plant contained diverse polyphenol with different monomeric constituents, linkages, relative and absolute concentrations etc., and their effect on the dentin matrix depended on the chemical composition and concentrations of these complex phytoconstituents.

Preserving the structural integrity of collagen, within the hybrid layer unimpregnated by resin, is crucial for the durability of resin dentin bonds. Polyphenols, including flavonoids, play a vital role in preserving the demineralised collagen matrix and increase the resin-dentin bond strength over time. Flavonoids, (a subgroup of polyphenols) found in plant derived cross linkers possess amphiphilic properties and interact physically with collagen, promoting cross linking and stabilising the collagen network, thereby reducing biodegradation [27]. Chung et al. [28] demonstrated that for the collagen to be cleaved, it has to be primarily unwound to allow the enzymes' active site to cleave individual chains. Thus, the role of cross linkers is very vital. The formation of exogenous collagen cross links improves the resistance of the collagen matrix to enzymes . Therefore, biomodification of dentin with collagen cross linkers contributes to a secured matrix network and a stable hybrid layer in dentin bonding. Dentin when treated with natural agents (plant-derived) such as proanthocyanidin, hesperidin, and EGCG have shown increased collagen resistance to degradation even when challenged with bacterial collagenases [29,30].

Polyphenols also chelate zinc, which is vital for maintaining functional, active sites of metalloproteinases and inhibiting MMP activity [31]. Consequently, high level of biocompatibility and effective collagen cross-linking capacity of polyphenols make them favourable MMP inhibitors. Also, Polyphenols, occurring naturally in plants, have been extensively studied for their capacity to cause MMP inhibition [32]. Plant flavonoids in whole plant extracts have also been investigated and employed topically for inflammatory skin disorders for MMP inhibition [33].

Thus, an attempt was made to preserve the integrity of the exposed collagen within the hybrid layer by evaluating the efficacy of 2 natural extracts, *Moringa Oliefera* and *Centella Asiatica*, rich in polyphenols, as collagen cross-linkers and MMP inhibitors on dentin's microtensile bond strength with resin composite. The results indicated that both the natural extracts at higher concentrations, acted as efficient cross-linkers stabilising the resin dentin bond (The bond strength values remained unchanged even after aging). *Moringa,* in particular, demonstrated superior performance stabilising the bond even after aging and exhibiting increased bond strength after

6 months (Figure 3) with a 5% concentration, which could be attributed to its cross linking property. This study utilised a universal bonding agent in the etch and rinse mode, following a systematic review [34], suggesting this technique improves microtensile bond strength.

Microtensile bond strength test is currently recommended for assessing the retention of resin composite restoration, especially after subjecting the specimens to a durability challenge [24]. It is calculated as the tensile load at failure divided by the cross-sectional area of the bonded surface. Though the preparation of micro specimens is a technique-sensitive, μTBS offers versatility, allowing multiple samples to be obtained from a single tooth. This test leads to more inventive study setups, better-controlled substrate variables, and economical use of teeth [24]. The test provides valuable information about biodegradation mechanism in in-vitro studies, even though these conditions may not occur separately in an intraoral condition. In this study, the most appropriate statistical approach was to conduct an unpaired T-test due to the nature of micro tensile testing, where the initial sample was destroyed and not reused after ageing.

It is worth noting that the type of MMP inhibitor, its concentration, and aging period can influence the impact of MMP inhibitors on bond strength [35]. Hence, this study examined the impact of MMP inhibitors on μTBS for up to 6 months utilising different concentrations cross linkers. Storage in water to simulate aging is one of the most common artificial technique to predict the behaviour of resin-based restorative materials [36] because the presence of water is critical for their deterioration of material [37]. To expedite the aging process, the specimens are sectioned into sticks, reducing the time required for water diffusion through the exposed resin dentin interface [38,39] during durability testing. In this study, artificial saliva was used as aging media. Another method used to study aging and bond durability is thermocycling [40], a thermal fatigue test which simulates thermal changes in the oral cavity caused by eating, drinking, and breathing [41]. The alteration in the temperature induces tedious contraction/expansion stresses at the tooth-material interface [5], resulting in crack propagation along the bonded interface.

The μTBS of 5% *Moringa* reported in this study was comparable with the results of Saffarpour et al. [42], who reported almost similar μTBS with chlorohexidine. Zheng et al. [43] documented slightly more μTBS with dentin treated with chemical MMP inhibitors, such as chlorohexidine and Galardin. Nagpal et al. [44] compared the influence of natural and synthetic matrix metalloproteinase inhibitors on dentin collagen preservation and their effect on long-term bond strength using total-etch adhesive. They reported that the natural MMP inhibitors increase the bond strength by improving resistance to collagen degradation. Al-Ammar et al. [18] concluded that glutaraldehyde and grape seed extract promoted chemical modification to the dentin matrix but not genipin when tested using μTBS.

With the addition of MMP inhibitors into the adhesive system may improve the bond strength and reduce the number of clinical steps. However, this may have a negative impact on other characteristics like degree of cure, elastic modulus, water sorption, and solubility [45]. During the incorporation process, consideration must be given to the stability and sustainability of the additional agents (MMP inhibitors) in the adhesive system. The MMP inhibition capacity of the agent will not be represented in the bond strength if non-polymerizable agents without covalent bonding capability are introduced to the adhesive, and the only action that manifests is delaying but not preventing the collagen degradation in the bond interface [46]. Therefore, in the present study, the natural MMP inhibitors were applied directly on the etched dentin surface.

Fracture mode analysis revealed no variation in adhesive or cohesive failure, among all groups. After aging, *Moringa* 5% showed 3 cohesive failures in resin composite, whereas the control group had only adhesive failures. The SEM analysis of the fractured fragments indicated a shift in the weakest sites within the resin dentin bond complex, aligning with previous study [12]. Fractures were more likely to occur in the adhesive layer or resin composite, rather than dentin or at the bottom of a hybrid layer after cross linker treatment. Although this observation was not statistically significant, it suggests that the weakest site after cross linking treatment was not within dentin.

In summary, the bond between resin and dentin became more stable after bio modifying

the dentin with 5% and 1% *Moringa*, even after aging. *Centella*, on the other hand, did not prove as effective in inhibiting MMP compared to *Moringa*. Therefore, using a pretreatment liner consisting of 5% *Moringa* can effectively inhibit MMP and cross-link collagen, resulting in improved bond strength. It is important to note that this study was conducted in vitro, and further research is necessary to evaluate the performance of these natural agents in vivo conditions.

The findings of the study have important implications for future research and clinical practice in several ways. Firstly, they highlight the potential of natural extracts as alternative MMP inhibitors, for more safer and biocompatible adhesive systems.

Secondly, this study underscores the importance of collagen cross linking in improving the durability of resin dentin bonds.

Third, these findings encourage further research into specific mechanisms by which natural extracts exert their MMP inhibition and collagen cross linking effects.

Author's Contributions

LA: Conceptualization, Methodology, Conceptualization, Methodology, Supervision, Writing – Original Draft Preparation. SR: Conceptualization, Formal Analysis, Resources, Data Curation, Visualization. VSKK: Writing – Review & Editing, Project Administration and Funding Acquisition. RKA: Software, Validation, Writing – Review & Editing. KG: Resources, Data Curation, Investigation, Resources.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of institutional Ethical committee of Narayana Dental College and Hospital. The approval code for this study is IEC/NDCH, 2019/P-24.

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