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VIABILITY OF USING NATURAL EXTRACTS IN DENTAL RESTORATIVE TREATMENT

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ABSTRACT

Objectives: Natural extracts can modify the dental surface to improve bond strength. This study evaluates the bond strength of restorative material to dentin treated with natural solutions. Methods: Ninety fragments of bovine dentin were etched with 35% phosphoric acid and divided according to the natural solutions: GI - Untreated (control), GII - *Camellia sinensis* (green tea), GIII - *Punica granatum* (pomegranate), GIV - *Vitis vinifera* (grape seed), GV - *Lycium barbarum* (goji berry). Sixty Specimens were used on the microtensile bond strength test (μTBS) were restored and sectioned ± 1mm^2 sticks (n=12). For adhesive interface analysis, fifteen fragments were treated with solutions, restored and prepared for SEM (n=3) and others fifteen fragments destined for surface analysis were treated with solutions and prepared for SEM (n=3). Results: Data were submitted to ANOVA and Tukey test (α=0.05). The highest values of μTBS ($p<0.05$) were obtained in the control groups (25.69 ± 6.32a), *Camellia sinen*is (28.14 ± 7. 66a) and *Vitis vinifer*a (25.55 ± 4.00a), which are similar to each other ($p>0.05$). The fragments treated with *Punica granatu*m (17.42 ± 4.82b) showed lowest values of μTBS ($p <0.05$). Fragments treated with *Lycium barbarum* showed intermediate values (23.44 ± 6.78ab) ($p>0.05$). SEM analysis of the dentin demonstrated a regular surface, without smear layer and the interface showed good hybridization of the entrance of dentin tubules and resin *tags*. Conclusion: The extracts solutions of *Camellia sinensis*, *Vitis vinifera* and *Lycium barbarum* did not interfere negatively on the bond strength of restorative material to dentin.

KEYWORDS: Adhesion; Dentin; Plant extracts; Scanning electron microscopy.

INTRODUTION

Natural extracts have been used in Restorative Dentistry to improve the stability of collagen and inhibit the activation of metalloproteinases (MMPs).[1,2,3] Prior to the restorative procedure, the phosphoric acid exposes the fibrils from the collagen matrix, allowing the infiltration of resin monomers in the interfibrillar spaces to create a mechanical retention of the restorative material to the dental substrate.[4,5] To occur proper adhesion, the substrate needs some humidity to promote the impregnation of monomers into dentin tubules and encapsulation of collagen fibrils by the adhesive.[6,7] The insufficient impregnation on dentin may cause an exposure and disorganization of collagen fibers[5], reducing the bond strength of the restorative material to dentin.[8] Studies have proposed the dentin pre-treatment with natural extract solutions to improve the stability of the matrix and induce the crosslinking of collagen fibers.[3,5,9]

Green tea is extracted from the leaves of the *Camellia sinensis* plant[10,11] and has about 4.000 bioactive compounds, mainly composed of polyphenols (catechins and flavonoids), gallic acid and phenolic acids (chlorogenic and caffeic).[11,12] Epigallocatechin-3-gallate (EGCG) is the most active and abundant catechin of green tea [11,13,14] with antioxidant and anti-carcinogenic properties, which directly scavenge and remove the excess of reactive free radicals.[10,11] Besides, green tea has been associated to anti-inflammatory and antimicrobial activity.[11] In Dentistry, it is used on caries prevention[15], to decrease loss of dentin in erosive and abrasive processes [16], to reduce halitosis[15] and also to improve interfacial bonds though inhibitory activity of MMPs.[13,14]

Pomegranate tree (*Punica granatum*) produces a fruit with gem-like seeds on the inside. It has polyphenolic flavonoids that have antioxidant, anti-inflammatory and antibacterial[17,18,19], antifungal and antiviral activity.[18,20] There is good evidence that pomegranate extract can

help treat bleeding disorders, respiratory diseases and hypertension. [17]

The grape extract is derived from the seeds of *Vitis vinifera* [21], has low toxicity [22] and mainly consists of proanthocyanidin [21,22], which are bioflavonoids [23] that interact with protein-rich in proline, such as collagen [1]. Grape seed extract has antioxidant, anti-microbial, anti-inflammatory and anticancer properties [24]. In dentin, it decreases biodegradation rates [25,26], improves the mechanical properties of the organic matrix [3,25] and has the ability to induce crosslinks in collagen matrix. [8,27]

Goji Berry (*Lycium barbarum*) contains polysaccharides, amino acids, carotenoids and flavonoids. [28,29] Its components have various biological activities, such as nutritional [30], neuroprotection, immunomodulation[30,31], cytoprotective, effects on aging [30], antioxidants [30,31], anti-carcinogenic, hypoglycemic [30], inflammatory properties and to the glaucoma treatment. [31] To date, there are only one study using *Lycium barbarum* extract in dental field that assessed the growth of human gingival fibroblasts to root surfaces. [32]

Considering that the components of natural extracts can modify the dentin collagen fibers and rehydrate the demineralized surface by phosphoric acid, the aim of this study was to evaluate the bond strength of restorative material to dentin and interface morphology of dentin pretreated with different natural extracts solutions: *Camellia sinensis*, *Punica granatum*, *Vitis vinifera* and *Lycium barbarum*.

MATERIALS AND METHODS

Experimental design

The study was conducted in a randomized design and considering the dentin treatment with natural extracts solutions at 5 levels: no treatment (control), *Camellia sinensis* (Green tea), *Punica granatum* (pomegranate), *Vitis vinifera* (grape seed) and *Lycium barbarum* (goji berry). Sample was composed by 90 intracoronary fragments of bovine dentin, in which 60 fragments were used in quantitative analyses by bond strength test (n=12), 15 in the qualitative analysis of the dentin surface by SEM (n=3) and 15 in the qualitative analysis of the adhesive interface by SEM (n=3). The schematic drawing of experimental design of this study are shown in Figure 1.

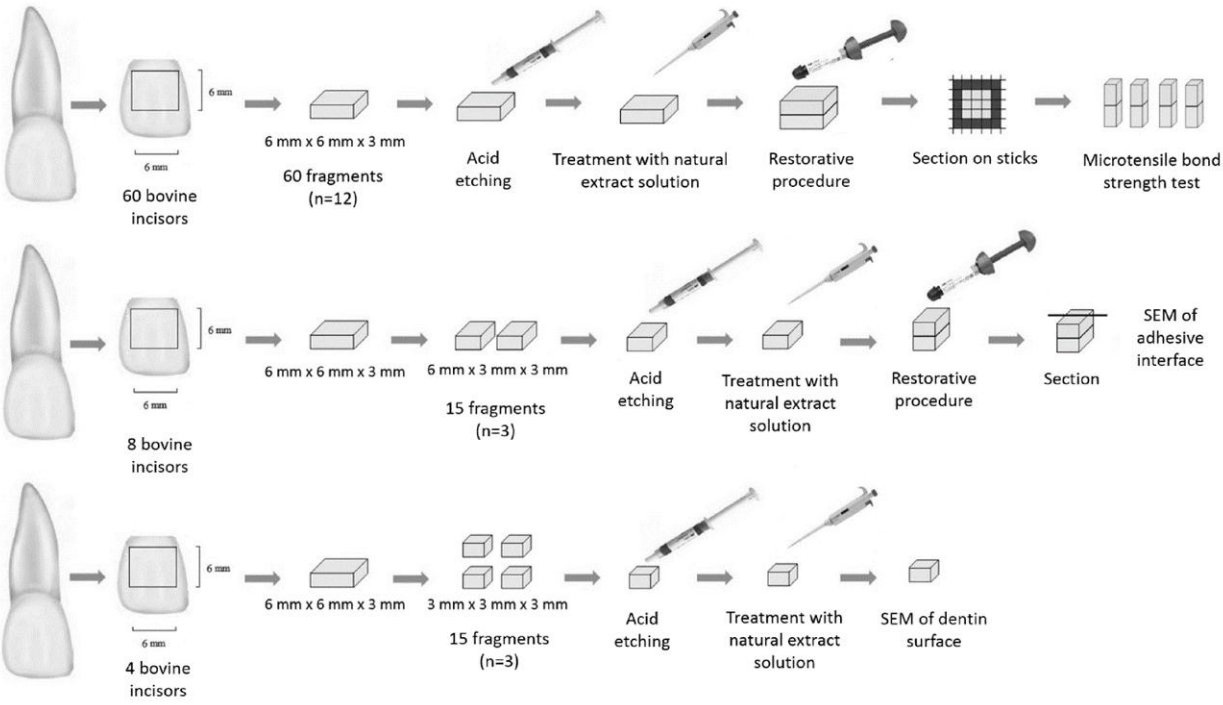

Figure 1. Schematic drawing of experimental design

Sample selection and preparation

Seventy-two extracted bovine incisors were stored in 0.1% thymol solution at 9°C and rinsed in tape water for 24 hours to complete eliminate the thymol residues and then, were examined with stereomicroscope (Leica Microsystems, Wetzlar, Germany) under x20 magnification. The incisors were sectioned transversally in cementoenamel junction to separate crowns from roots, longitudinally separating the buccal and lingual surfaces and subsequently, the buccal surface of each crown was sectioned to obtain dentin slabs, using a slow-speed water-cooled diamond saw (Isomet 1000; Buehler, Germany), obtaining 72 fragments of 6 mm height x 6 mm wide x 3 mm thickness. The dentin surfaces of specimens were flattened in a polishing machine (Arotec, Cotia, SP, Brazil) with 600-grit sandpaper (Norton; Lorena, SP, Brazil) for 15 seconds in order to standardize the smear layer.

Eight dentin slabs used in the analysis of the adhesive interface by SEM were cut in half, to obtain 16 specimens of 6 mm high x 3 mm wide x 3 mm thickness, and to the dentin surface analysis, 4 fragments were cut in order to obtain 15 specimens of 3 mm high x 3 mm wide x 3 mm thickness.

Solutions preparation

The solutions were prepared using the powder of each extract (Aurea Pharma, Ribeirão Preto, SP, Brazil) and distilled water [16,33], at a concentration of 0.5%, standardized in a preliminary study. The extracts were weighed on an analytical balance and the distilled water was added. The solution remained in a mixer for 15 minutes at 70°C, followed by ultrasonic for 15 minutes and thereafter, mixer again for 15 minutes. After preparation, the solutions were subjected to centrifugation (Mini Hight Speed Centrifuge, Topscien Instrument, Ningbo, China), for 2 minutes at speed of 11 rpm and subsequent separation of liquid from precipitate (defined by preliminary study).

Surface treatment

The application of 35% phosphoric acid (Scotchbond, 3M ESPE, St. Paul, MN, USA) was performed for 15 seconds on the dentin surface of all fragments, followed by washing with distilled water for 30 seconds and drying with absorbent paper. Then, the specimens were randomly divided into 5 groups according to the experimental solutions: GI - untreated (control), GII - *Camellia sinensis* extract solution (green tea), GIII - *Punica granatum* extract solution (pomegranate), GIV - *Vitis vinifera* extract solution (grape seed), GV - *Lycium barbarum* extract solution (goji berry). It was applied 1 mL of each solution on dentin surface with micropipette and remained in contact with the substrate for 1 minute. Then, the solution was removed with aspirating cannula and the slabs were dried with absorbent paper.

Analysis of dentin surface by SEM

After the dentin treatment, 15 slabs were immersed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.4 at 4°C temperature for 12 hours, followed by washing in distilled water for 3 minutes and immersion in distilled water for 1 hour, with changes every 20 minutes. Then, the specimens were dehydrated using increasing concentrations of ethanol of 25°, 50°, 75°, 95° GL, with ethanol change every 20 minutes intervals, subsequent immersion for 1 hour in ethanol 100° GL, and finally, were immersed in HMDS for 10 minutes to obtained chemical drying of the fragments, dried and stored at 37°C for 24 h.

After this period, they were fixed in stubs with carbon tape and analyzed under scanning electron microscope (SEM; S3400N, Hitachi, Tokyo, Japan) and the most representative area of each group at different magnifications was photographed.

Restorative procedure

It was applied two adhesive layers (Single Bond 2 3M ESPE, St. Paul, MN, USA) on the dentin surface according to the manufacturer. Three 1-mm thick layers of composite resin (Filtek Z250 XT, 3M ESPE, St. Paul, MN, USA) was placed over the bonded dentin and polymerized using LED light source (Gnatus, Ribeirão Preto, SP, Brazil), for 20 seconds at 1200 mW/cm^2, with the intensity of the LED measured with curing radiometers, to obtain a resin block with dimensions of 6 mm height x 6 mm wide x 3 mm thickness. After the restorative procedure, specimens were stored in distilled water at 37°C for 24 hours. The 60 specimens restored for μTBS were sectioned to obtain 4 sticks with a cross-sectional area of approximately 1.0 mm^2 from each specimen, selected from the central area, excluding the extremities.

Microtensile bond strength (μTBS) test

The dimensions of each stick was measured using a digital caliper and the resin/dentin interface was positioned at the free space between the custom-made testing jig of the universal testing machine (Instron Corporation, Canton, MA, USA) using a cyanoacrylate glue (Super Bonder Original, Henkel Ltda., São Paulo, SP, Brazil), and subjected to a tensile force with a 50 kgf load cell and a crosshead speed of 0.5 mm/min, until failure. μTBS was expressed in megapascals (MPa), provided by the program, calculated via the following equation: performed (N)/ bonding area (mm^2).

The surfaces involved in the failure of each specimen were analyzed by confocal scanning microscope 3D laser (LEXT OLS4000®, Olympus Corporation, Japan) (objective lens 20x). Failures patterns were classified in adhesive, when the dentin surface was covered by a thin layer of adhesive material; cohesive material, when the surface was covered with composite resin; cohesive substrate, when failure occurs in the dentin; and mixed, in situations where there is a combination of adhesive and cohesive type.

Analysis of adhesive interface by SEM

Fifteen specimens were restored as previously described and were sectioned into two halves. The sections were ground with 600- and 1.200-grit sandpapers (Norton; Lorena, SP, Brazil) for 30 seconds each and with alumina 0.3 μM and 0.05 μM for 5 minutes.

After polished, the sections received 35% phosphoric acid (Scotchbond, 3M ESPE, St. Paul, MN, USA) for 20 seconds, followed by rinsing at the same time and then were ultrasonically cleaned for 10 minutes. Then, the sequence of preparation was performed as described for the analysis of dentin surface by SEM, and the specimens were stored at 37°C for 24 h.

After this period, the specimens were visualized by gold sputtering (Bal-Tec SCD 005 Sputter Coater, Balzers, Liechtenstein) and analyzed under SEM (EVO 50; Carl Zeiss, Cambridge, England). The adhesive interface was

analyzed qualitatively and was observed the presence and uniformity of the hybrid layer, the tags and possible gaps of the adhesive interface subjected to different dentin pre-treatments and the most representative area were photographed.

Data analysis

Data of the microtensile bond strength test were analyzed using the Statistical Package for Social Sciences software for Windows (SPSS 19, SPSS Inc, Chicago, IL, USA), and the groups were compared to verify the differences at a pre-set alpha of 0.05, showing normal and homogeneous distribution (Kolmogorov-Smirnov and Bartlett test). Then, they were analyzed by ANOVA one-way and Tukey test ($p < 0.05$).

RESULTS

Microtensile bond strength test

Data analysis showed significant differences among groups ($p=0.0013$). The μTBS mean values and standard deviation of the different experimental groups are shown in Table 1.

The specimens treated with with *Camellia sinenis* extract (Green tea, GII) showed higher μTBS values, similar to other groups ($p>0.05$), except for dentin fragments treated with the *Punica granatum* extract ($p<0.05$) (Pomegranate, GIII). Fragments that received *Lycium barbarum* extract showed intermediate values (Goji Berry, GV) ($p> 0.05$), statistically similar ($p>0.05$) to all experimental groups.

Table 1. Means values of bond strength and standard deviation of the experimental groups

Experimental groups	Mean and standard deviation
GI – Control, untreated	25.69 ± 6.32a
GII – *Camellia sinensis* extract	28.14 ± 7.66a
GIII – *Punica granatum* extract	17.42 ± 4.82b
GIV – *Vitis vinífera* extract	25.55 ± 4.00a
GV – *Lycium barbarum* extract	23.44 ± 6.78ab

* *Different letters indicate significant statistical difference. Tukey test ($p<0.05$).*

Considering the failures pattern, it was observed the predominance of adhesive failure in all groups. The specimens treated with *Lycium barbarum* solution resulted in an increase in mixed failure rates, compared with other groups (Figure 2).

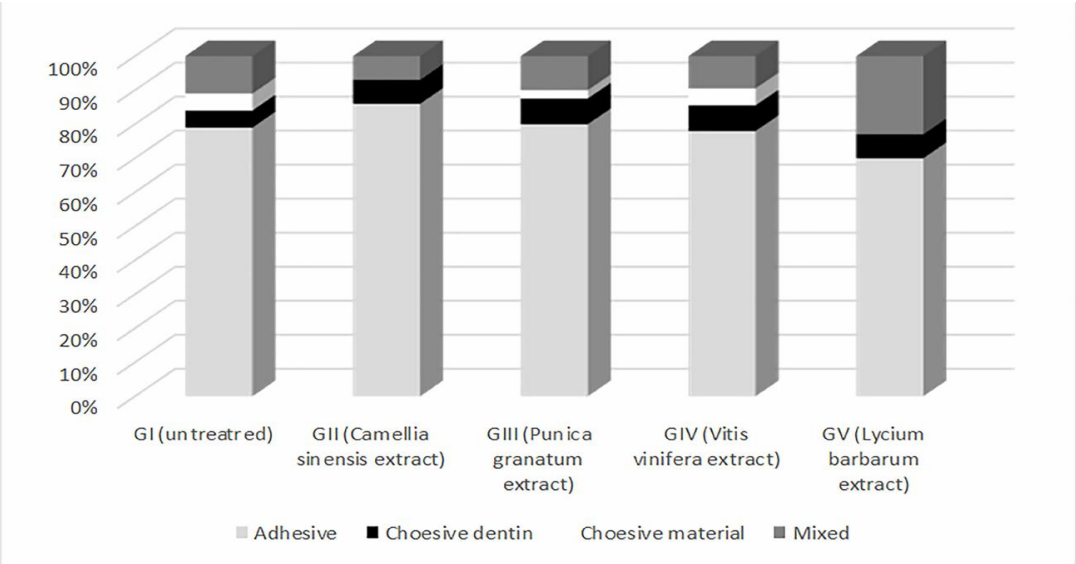

Figure 2. Graphical representation of percentages failure pattern of the different experimental groups.

Morphology of the dentin surface

After application of the natural extracts solutions on the dentin previously treated with 37% phosphoric acid, it was observed homogeneous surface of demineralized dentin, without smear layer and opened dentinal tubules in all analyzed groups. The fragments treated with *Punica granatum* extract had some granules inside dentinal tubules, possibly resulting from residues of the solution-(Figure 3).

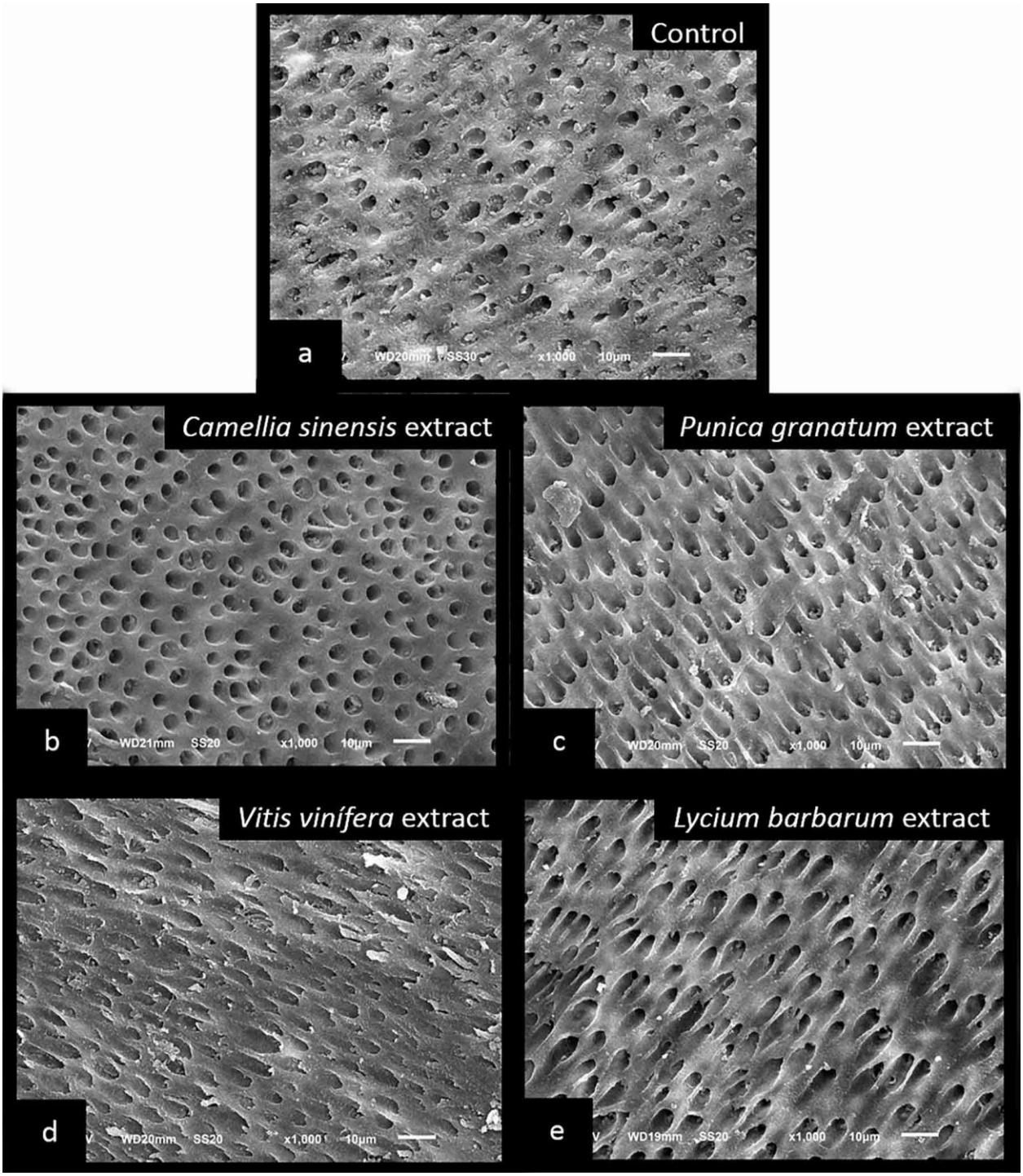

Figure 3. **Photomicrographs (1000x) representative of the dentin surface treated with 37% phosphoric acid followed by rewetting with natural extracts: (a) GI – Untreated (control); (B) GII - *Camellia sinensis*; (C) GIII - *Punica granatum*; (D) GIV - *Vitis vinifera*; (E) GV - *Lycium barbarum*.**

Morphology of the adhesive interface

It was observed that in all groups had complete filling of the hybrid layer, hybridization on the dentinal tubules and good resin infiltration with resin tags formation.

In dentin fragments treated with *Punica granatum* solution (GIII), it was verified possible granules inside the dentinal tubules. The fragments treated with *Lycium barbarum* solution (GV) had slight and less homogeneous formation of the hybrid layer, compared to the other groups. (Figure 4).

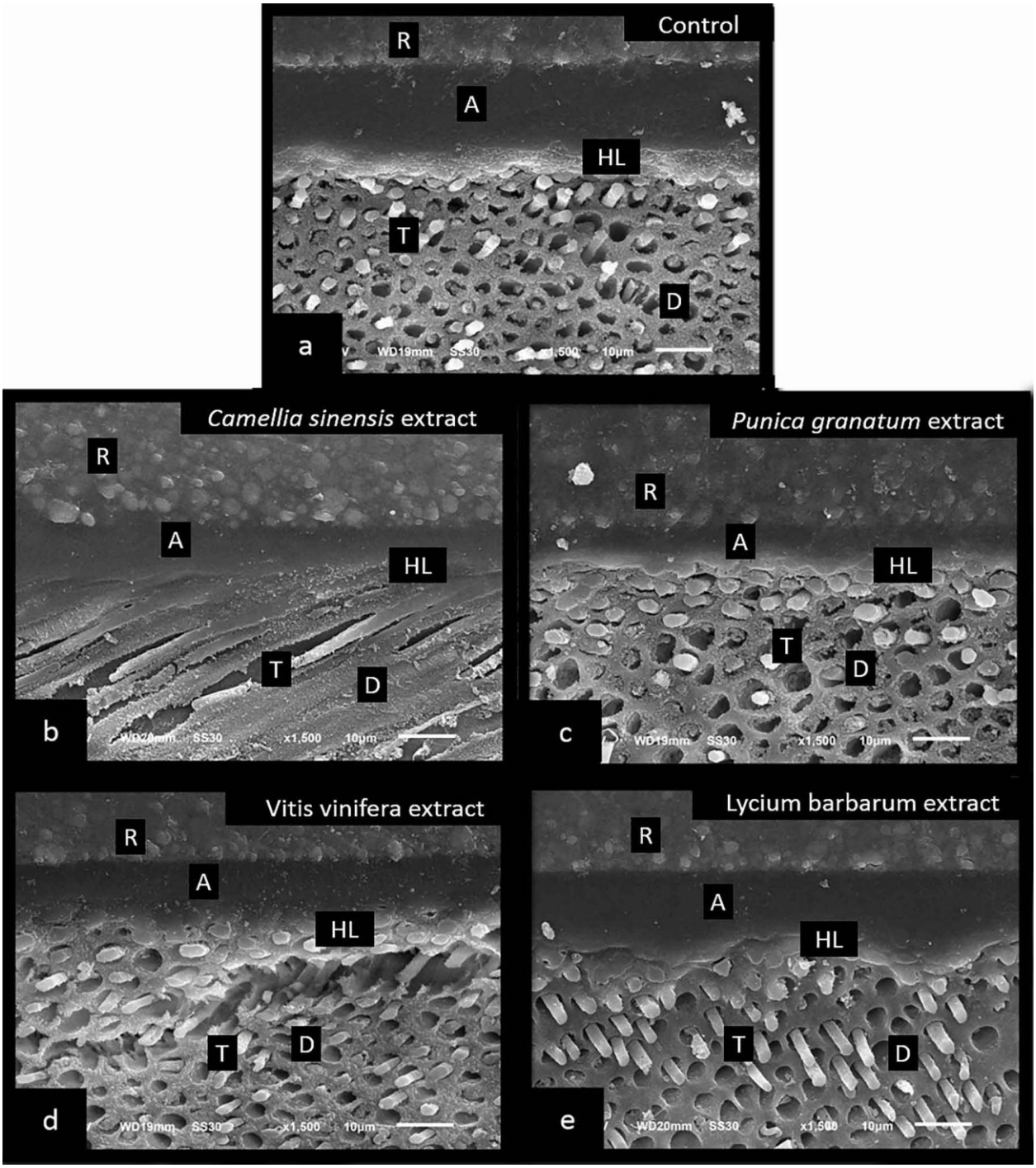

Figure 4. **SEM images, representing the adhesive interface of each group: (a) GI - untreated group (control); (B) GII - *Camellia sinensis*; (C) GIII - *Punica granatum*; (D) GIV - *Vitis vinifera*; (E) GV - *Lycium barbarum*. R = Resin, A = adhesive, HL = hybrid layer, t = *tags*, D = Dentin.**

DISCUSSION

Recent literature has highlighted the favorable properties of natural extracts in Dentistry. [2,5,9,10,19,20,34] These solutions can rehydrate the demineralized dentin by phosphoric acid, protect collagen fibers and allow complete penetration of resin monomers in the collagen matrix and dentinal tubules. [6,7] However, little is known about the viability of using these solutions to increase the bond strength of the restorative material to the tooth substrate.

This study used the phosphoric acid in dentin prior to application of the solutions. It is assumed that the natural extracts solutions can hydrate the demineralized dentin surface and increase the amount of cross-linked collagen matrix in order to prevent fiber collapse. Thus, it would increase immediate bond strength and resistance to degradation of adhesive interface. [35]

The outcomes of this study showed that *Camellia sinensis* and *Vitis vinifera* extracts applied on dentin promoted bond strength similar to untreated substrate (only etched with phosphoric acid), and these specimens did not differ from those treated with *Lycium barbarum* extract.

Studies showed superior results of *Camelia sinensis* in bond strength when compared to untreated substrate.[11,13,14] However, these studies did not used green tea extract but the pure EGCG. This catechin has inductive effect of crosslinking on dentin collagen with stabilization of triple helix molecule that provides higher strength of fibrillar matrix. [13,14,36] Besides, EGCG prevents the free access of collagenase to active sites on the collagen chains, thus might has an inhibitory effect of collagen degradation. [36]

The grape seed extract (Vitis vinifera) is also able to induce crosslinking in dentin collagen matrix [8,22,27] increasing the matrix stiffness. [27] The crosslinking mechanism between proanthocyanidins and collagen it is not well-defined [37], and four interactions are proposed: covalent bonds, ionic bonds, hydrogen bonds and hydrophobic interactions. [22,23] It is believed that bioflavonoids can induce additional intra and intermolecular bonds of collagen [23], and thus improve the mechanical properties of the dentin. [3,22,23,37]

Previous studies [5,9,22] verified that grape seed extract act as a modifier of collagen matrix, improving strength unity and stability of the dentin. One possible explanation for such differences is the fact that these studies used concentrations of 6.5% [5,9,22] and raging from 5 to 15% [34] and our study used 0.5% for all solutions.

Specimens treated with *Lycium barbarum* extract (goji berry) had intermediate values of bond strength. The extract did not affect the bond strength when compared to untreated specimens (control), and was similar to *Camellia sinenis* and *Vitis vinifera*.

Among the chemical constituents of *Lycium barbarum* fruit stands out polysaccharides, which are estimated to comprise 5–8% of the dried fruits, carotenoids, betaine, cerebroside, beta-sitosterol, p-coumaric acid and vitamins. [38] Betaine have ability to increases memory, relieve anxiety, muscle growth and protects against fatty liver illness. Carotenoids have effective in preventing cardiovascular diseases and skin cancer. The major active compound of goji berry is the *Lycium barbarum* polysaccharide (LBP) [29,39] that have antioxidant activity[29], increasing immune capacity and reducing blood glucose [29]. However, to date, only one study with *Lycium barbarum* extract was found in dentistry that investigated its effect in periodontal tissue. [32]

The specimens treated with *Punica granatum* extract (pomegranate) showed the lowest bond strength, similar to those treated with the *Lycium barbarum*.

The polyphenols present in fruits of *Punica granatum* are predominantly ellagitannins, hydrolysable tannins that release ellagic acid on hydrolysis. [40] Punicalagin is responsible for more than 50% of antioxidant activity of the fruit and is more potent than EGCG, quercetin and curcumin, in the ability to inhibit MMP-13 activity. [40] Recent studies showed that this extract increases the amount of collagen in osteoblasts [41] and inhibited bovine cartilage degradation. [40] The polyphenols are able to increase resistance of collagenase degradation due to induction of hydrogen bonds with free amino acids in collagen fibers. Hydrophobic and electrostatic interactions can be classified as an additional source of stabilization. [40] To date, there are no studies using *Punica granatum* extract in bond strength. Dentistry studies suggest the efficacy of *Punica granatum* in the control of oral pathogens responsible for caries [20], periodontal diseases [20,42], biofilm formation on orthodontic wires [43], antibacterial and antifungal action[20], and as a storage medium for avulsed teeth. [17]

In specimens treated with green tea and grape seed extract can be observed under SEM homogeneous hybrid layer and resin tags, similar to control group. [34,44] Dental fragments treated with *Punica granatum* extract showed some granular residues within dentinal tubules and on surface that might influenced negatively the bond strength of restorative material to dentin. Others extraction methods of bioactive component can eliminate the granular residues if the solution is performed at higher concentration, may occur potentiation of the effects.

Despite the *Camellia sinensis*, *Vitis vinifera* and *Lycium barbarum* extracts did not increase the bond strength compared to untreated specimens, these solutions do not interfere negatively in the immediate bond strength of restorative material to dentin. For this fact and considering the above-mentioned positive properties, a promising potential of these natural solutions as dentin biomodifier cannot be discard.

This study opens perspectives to assess other extraction protocols of bioactive compounds at different concentrations and application mode. Our research group is evaluating the effect of natural extracts solutions as inhibitors of collagen degradation. Overtime, these new adhesive protocols has the potential to improve long-term durability of interface and can be adjuvant approach for remineralization of dental substrate. Further, investigations are also necessary to understand the therapeutic potentials on Dentistry.

CONCLUSIONS

According to the methodology and the results obtained in this study, it may conclude that:

1. Natural extracts solutions of *Camellia sinensis*, *Vitis vinifera* and *Lycium barbarum* did not affected the bond strength of restorative material to dentin.
2. *Punica granatum* solution decreased the bond strength of the restorative materials to dentin.
3. Natural extract solutions provided good interface morphology with resin tags, similar to control group.

Declaration of Conflicting Interests

The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

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