RESEARCH PAPER

Antioxidant and Antimicrobial assessment of Green Tea Incorporated Dentin Bonding Agents - An In Vitro Study

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ABSTRACT

Background: Recent research has focused on incorporating natural bioactive compounds into dentin bonding agents to enhance their biological availability. Green tea polyphenols, particularly epigallocatechin-3-gallate (EGCG), are known for their strong antioxidant and antimicrobial effects and may play a significant role in preserving the integrity of the resin-dentin interface.

Aim: To assess the antioxidant and antimicrobial efficacy of green tea incorporated dentin bonding agents.

Materials and Methods: This in vitro experimental study was conducted in the Orange lab, Saveetha Dental College and Hospitals, Chennai, India, from January to March 2025. Green tea extract was incorporated into a total-etch and a self-etch dentin bonding agent. Antioxidant activity was measured by the DPPH free radical scavenging assay method and was measured at concentrations from 10 to 100 µL, using ascorbic acid as the positive control. Confirmation of polyphenolic compounds was done on UV–visible spectrophotometry at 274 nm. The antimicrobial action against Streptococcus mutans was checked using the agar diffusion method by measuring zone of inhibition. Each experiment was done in six independent replicates, n = 6. After testing for normality using the Shapiro–Wilk test, one-way ANOVA was performed, followed by Tukey post-hoc test; two-way ANOVA was used for dose-dependent testing and Welch's t-test for paired testing. The level of significance was considered $\alpha = 0.05$.

Results: Green tea-containing adhesives revealed marked antioxidant activity and zones of inhibition in a dose-dependent manner. The self-etching adhesive showed a higher antioxidant activity and zone of inhibition when compared with the total etch system in a statistically significant manner ($p < 0.001$)

Conclusion: Green tea incorporation enhanced the biological performance of dentin bonding agents, with the self-etch approach demonstrating enhanced antioxidant and antimicrobial activity, supporting its potential for using it as a dental adhesive technique.

Keywords: Dental Bonding; Green Tea; Antioxidants; Anti-Bacterial Agents; Streptococcus mutans.

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.INTRODUCTION

For successful adhesive restorative dentistry in the long term, it is not sufficient to focus solely on efficient bonding but must address the need for dentin bonding agents to have resistance to bacterial adhesion and oxidative degradation at the resin dentin interface. Although research in dental adhesives continues to improve, secondary caries remains a major factor in successful restoration outcomes in caries active patients, such as children and teenagers.[1] This underlines a need for research into dental adhesives with additional biological properties.

The primary objective of current dentin bonding agents, including total-etch and self-etch systems, is to achieve optimum hybrid layer formation. However, due to the lack of inherent antibacterial or antioxidant properties in these materials, bacteria such as Streptococcus mutans can survive at the site of restorations and induce recurrent caries.[2] Moreover, over time, the durability of the bond may be negatively affected by oxidative stress and degradation of resin components that result from polymerization.[3] Therefore, integrating bioactive components into adhesive systems has emerged as a viable strategy for enhancing the biological and functional durability of restorative materials.

The polyphenol-rich extract from green tea is mainly composed of catechins, especially epigallocatechin gallate, which is known for its antioxidant, antibacterial, and anti-inflammatory properties. Green tea polyphenols, in particular epigallocatechin-3-gallate, are known for their antioxidant and antimicrobial properties; however, few studies have reported how different adhesive approaches affect their retention and functional behavior. [4] EGCG plays an important role in cariology and preventive dentistry because it has

been shown to inhibit S. mutans growth and acidogenicity, as well as reduce biofilm formation.[5] Moreover, the powerful ability of green tea polyphenols to scavenge free radicals could reduce oxidative stress and improve resin stability at the adhesive interface.[6]

Green tea extracts have already been investigated in various dental products such as mouthwashes, toothpaste, and filling materials, showing promising antimicrobial and antioxidant properties.[7, 8]. Nevertheless, most studies have focused on the biological properties of green tea rather than focusing on the combined effect of incorporating with dental adhesive. Few studies have tried to incorporate green tea or EGCG into dentin bonding materials, and majority of these studies have concentrated on antimicrobial and/or antioxidant properties.[9] Also, literature on the comparative studies involving different adhesive systems such as total etch and self-etch is scarce.

The resin composition and hydrophilicity of adhesive systems significantly influence the dispersion, retention, and sustained release of bioactive agents. Self-etch adhesives, owing to their relatively hydrophilic nature, facilitate better integration and availability of green tea polyphenols compared to conventional total-etch systems.[10] However, this assumption has not been sufficiently validated by further studies..

Interest in incorporating natural bioactive compounds into dental adhesives has been the focus of research in recent years due to their added biological benefits at the adhesive dentin interface, particularly in reducing oxidative stress and bacterial activity that contribute to secondary caries. Therefore, the present study was done to evaluate the antioxidant and antimicrobial evaluation of green tea incorporating total-etch and self-etch dentin bonding agents. By directly comparing two commonly used adhesives, this study addresses the gap in the existing literature and provides adequate evidence on the influence of adhesive agents on the biological performance of green tea modified dentin bonding agents, contributing to the development of bioactive adhesive systems with improvised preventive potential.

MATERIALS AND METHODS

This in vitro study was conducted at the Orange Lab, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Chennai, India, during the time period from January to March 2025. An in vitro comparative experimental design was followed to evaluate the antioxidant and antimicrobial properties of green tea incorporating total-etch and self-etch dentin bonding agents. Although the study did not involve human participants, institutional permission was obtained from the Saveetha Research Board (SRB Approval No: 9472487184787959) prior to commencement of laboratory procedures. The study protocol and reporting were carried out following the established guidelines for in vitro dental materials research, with significance on methodological standardization, reproducibility, and clear outcome reporting [11,12].

Preparation of Green Tea Extract

Fresh green tea leaves (Camellia sinensis) were procured from an authorized local commercial supplier in Chennai, Tamil Nadu, India and washed well with distilled water to remove surface impurities. Ten grams of shade-dried green tea leaves were extracted in 100 mL distilled water at 60°C for 30 minutes, filtered, and stored at 4°C. The leaves were shade-dried at room temperature and chopped fine to increase extraction efficiency. The processed leaves were subjected to aqueous extraction using distilled water, followed by filtration through Whatman No. 1 filter paper to obtain a clear extract. The prepared extract was stored in light-protected containers at 4°C until use to minimize degradation of polyphenolic compounds.

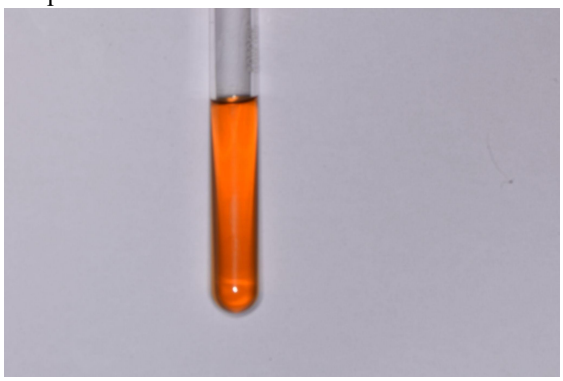

Figure 1: shows the green tea extract prepared for incorporating into dentin bonding agents

Two commercially available dentin bonding agents representing different adhesive strategies were evaluated: a total-etch adhesive incorporated with green tea extract (Group A) and a self-etch adhesive incorporated with green tea extract (Group B). Green tea extract shown in figure 1 was incorporated into each adhesive at a 1:1 volume ratio, based on preliminary bioactive adhesive screening studies. The 1:1 volume ratio of green tea extract to adhesive was selected to maximize the availability and detectability of polyphenolic bioactivity during antioxidant and antimicrobial screening assays. This concentration was intended for biological evaluation only and does not represent a clinically optimized adhesive formulation. Mixing was done under aseptic conditions using gentle agitation to realize homogenous dispersion without inducing premature polymerization, and all modified adhesives were freshly prepared immediately before testing to prevent phase separation.[8]

DPPH Free Radical Scavenging Assay

Antioxidant activity was quantitatively evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay at the Department of

Microbiology, Orange Lab, Saveetha Dental College. A 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of 99% methanol, with ascorbic acid (10 mg/mL) used as the positive control. The test samples were green tea extract (GT), self-etch adhesive incorporated with green tea (SE+GT), and total-etch adhesive incorporated with green tea (TE+GT). Aliquots ranging from 10 to 100 µL of each sample were dispensed into ELISA plate wells, followed by the adding 100 µL of DPPH solution to achieve a final volume of 200 µL per well, while methanol with DPPH served as the negative control. The plates were incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 517 nm using a microplate plate reader. The percentage of inhibition was calculated using the formula: Percentage of inhibition (%) = [(Absorbance of control − Absorbance of sample) / Absorbance of control] × 100, where control represents the DPPH solution without the test sample and test groups represent the DPPH solution containing the test material. All assays were performed in triplicate, and mean values were used for statistical analysis.[13,14]

Antimicrobial Activity Evaluation

The antimicrobial properties were tested against Streptococcus mutans(ATCC 25175), which is a major cariogenic bacterium involved in secondary caries. Standardized bacterial samples were obtained according to standard microbiology practices and were normalized at a 0.5 McFarland standard. The antimicrobial effectiveness was tested using the agar well diffusion technique, which is a widely recognized screening tool for testing the antimicrobial properties of dental materials. The sterile agar plates were evenly challenged with bacterial samples to achieve a confluent bacterial lawn, and wells with a 6-mm diameter were produced using a cork borer. A volume of 50-100 µL of each material, including green tea extract, green tea-integrated total etch adhesive, and green tea-integrated self-etch adhesive, were placed into each well. Methanol-treated wells and adhesive without green tea served as negative and solvent controls, respectively. The plates were incubated for 24 hours at 37°C, and subsequent measurements of the zone of inhibition were determined using digital vernier calipers after incubation. The measurements were undertaken by a single examiner to reduce inter-examiner error.[15,16]

Statistical analysis

Statistical analysis was performed after confirming data normality using the Shapiro–Wilk test, which demonstrated that all variables followed a normal distribution ($p > 0.05$). Accordingly, parametric statistical methods were applied, and data are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare antioxidant activity among groups at 100 µL concentration, while two-way ANOVA was employed to assess dose-dependent antioxidant effects. Antimicrobial efficacy between adhesive groups was analyzed using Welch's t-test. Effect sizes were calculated using eta-squared (η^2) for ANOVA and Cohen's d for t-test comparisons. Statistical significance was set at $p < 0.05$.

RESULTS

Group	% DPPH Inhibition, Mean ± SD	f value	p value
Ascorbic acid (positive control)	91.6 ± 2.3	217.8	<0.001
Green tea extract	81.7 ± 2.1		
Self-etch + Green tea	75.4 ± 2.0		
Total-etch + Green tea	68.2 ± 1.8		

Table 1: DPPH antioxidant activity (100 µL)

Highly significant differences in antioxidant activity were found among the four groups using a concentration of 100 µL in the DPPH assay as shown in table 1. One-way ANOVA showed a highly significant overall difference among the groups with a very large effect size ($F = 217.80$, $p<0.001$; $\eta^2 = 0.95$) pertaining to their free radical scavenging abilities. Post-hoc Tukey analysis showed a distinct ranking order in antioxidant activity with a higher potential observed in ascorbic acid, followed by green tea extract, self-etching green tea adhesive samples, and total etch green tea adhesive samples. Moreover, self-etching green tea samples showed significantly higher antioxidant activity than those in total etch systems ($p < 0.001$).

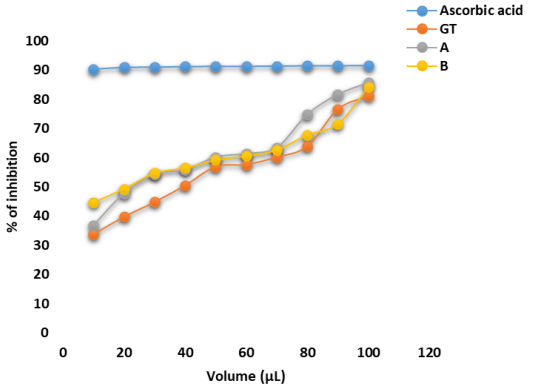

Figure 2: Dose-dependent DPPH radical scavenging activity (10–100 µL) of ascorbic acid,

green tea extract, self-etch + green tea, and total-etch + green tea.

Figure 2 shows a line graph illustrating dose-dependent DPPH radical scavenging activity (10–100 µL) of ascorbic acid, green tea extract, self-etch adhesive with green tea, and total-etch adhesive with green tea. All groups exhibited an increase in antioxidant activity with increasing concentration, with higher values observed for the self-etch green tea group compared with the total-etch system across the tested range.

Sample	10 µL	20 µL	40 µL	60 µL	80 µL	100 µL	P value
Ascorbic acid	35.2 ± 2.1	50.1 ± 2.4	65.8 ± 2.0	75.6 ± 1.9	84.2 ± 2.1	91.6 ± 2.3	<0.001
Green tea extract	30.4 ± 2.0	45.6 ± 1.9	60.3 ± 2.1	68.9 ± 1.8	75.1 ± 2.0	81.7 ± 2.1	
Self-etch + green tea	25.8 ± 1.9	40.2 ± 2.0	55.1 ± 2.2	63.4 ± 1.7	70.3 ± 1.9	75.4 ± 2.0	
Total-etch + green tea	20.6 ± 1.8	35.4 ± 2.1	50.2 ± 2.0	58.1 ± 1.6	65.0 ± 1.7	68.2 ± 1.8	

Table 2: Dose-dependent DPPH activity

The percentage of DPPH radical scavenging increased linearly with concentration from 10 to 100 µL for all tested samples as shown in table 2. Two-way ANOVA demonstrated that the main effect of sample type was highly significant ($p<0.001$), as was the effect of concentration ($p<0.001$), thus indicating that both the nature of the material and the applied concentration significantly affected antioxidant activity. Moreover, a significant sample × concentration interaction was noted at $p<0.001$, demonstrating that the magnitude of dose-response varied between the different samples. At each concentration tested, ascorbic acid maintained the highest ranking order in percentage of inhibition, followed by green tea extract, self-etch adhesive incorporated with green tea, and the total-etch green tea–incorporated adhesive. More significantly, the self-etch green tea group displayed higher antioxidant activity than did the total-etch system at every concentration tested, which validated superior dose-dependent antioxidant behavior.[15]

Group	Zone of Inhibition (mm), Mean ± SD	t	df	p value
Total-etch + Green tea	14.9 ± 1.1	11.78	11.67	<0.01
Self-etch + Green tea	19.8 ± 1.0			
Green tea extract	17.8 ± 1.2			
Negative control	2.1 ± 1.3			

Table 3: Antimicrobial activity of green tea–incorporated dentin bonding agents against Streptococcus mutans(ZOI).

Table 3 shows that all the green tea–containing groups had measurable antimicrobial activity against Streptococcus mutans, while the negative control group showed very slight inhibition. Among the tested adhesives, green tea–incorporated self-etch dentin bonding produced a significantly larger zone of inhibition than that of total-etch green tea–incorporated adhesive. The results showed that Welch's independent t-test revealed a highly significant difference between the two groups: $t = 11.78$, $df = 11.67$, $p<0.001$ (means difference = 4.84 mm, 95% CI: 3.95–5.74 mm). The magnitude of this difference was further indicated with a very large effect size, Cohen's $d = 5.55$, indicating a strong material-dependent antibacterial trend favoring the self-etch adhesive.

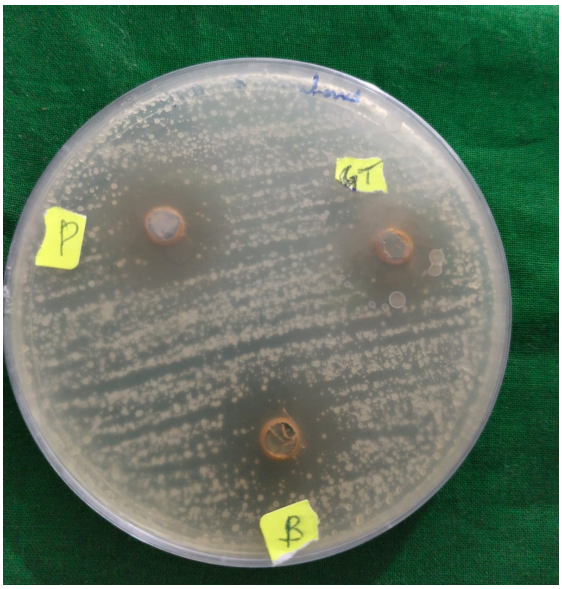

Figure 3: Antimicrobial activity (ZOI) against Streptococcus mutans

Figure 3 quantitative comparison of zones of inhibition produced by green tea extract, green tea–incorporated self-etch adhesive, and green tea–incorporated total-etch adhesive against Streptococcus mutans (ATCC 25175) using the agar well diffusion method. The self-etch green tea group showed a significantly larger zone of inhibition compared to the total-etch green tea group.

DISCUSSION

The study focused on the biological activity of green tea–incorporating dentin bonding agents regarding their antioxidant capacity and antimicrobial efficacy. The results indicate that the incorporation of green tea extract into adhesive systems enhances both free radical scavenging activity and antibacterial action against oral microorganisms. Interestingly, it was seen that the self-etch adhesive containing green tea showed consistently higher activities than the total-etch counterpart, highlighting the impact of adhesive resin on functional expression of bioactive components. Also, the 1:1 incorporation ratio was employed as a high-concentration screening model to evaluate biological potential, and its effects on polymerization kinetics, viscosity, and bond strength which were not assessed in the present study.

The increased antioxidant activity in this study is consistent with another study conducted by Du et al. [17] because they were able to show that epigallocatechin-3-gallate (EGCG) can be added to dental adhesives without inhibiting polymerization but with preserved antioxidant properties. To a great extent, Macedo et al. [18] arrived at a similar conclusion because they were able to demonstrate EGCG-modified adhesives with good physicochemical properties and resistance to oxidative degradation. Additionally, the dose-dependent increase in DPPH radical scavenging activity in this study corroborates with other studies conducted by Yang et al. [19] because they highlighted the capacity of EGCG to scavenge any existing free radicals in an adhesive.

The persistent higher antioxidant activity of the self-etch green tea-containing adhesive in all concentrations can be attributed to a different composition of the adhesive in interaction with the polyphenolic compounds. Self-etching adhesives mainly consist of hydrophilic resin monomers and an acid function, which can have a role in better solubilization of green tea catechins. This can be explained in light of the study conducted by Epasinghe et al which showed increased resistance to degradation of adhesive interfaces with increased concentration of green tea when incorporated into less aggressive adhesive systems.[9,20] Moreover, increased stability of the collagen present in the interface of adhesives and dentin with EGCG delivery systems by inhibiting matrix metalloproteinases has been evidenced by Yu et al. [21].

The antibacterial results of the present study provide further evidence for the biological relevance of incorporating green tea into dental adhesives. Whereas the negative control exhibited very limited inhibition, all experimental groups containing green tea showed measurable antibacterial activity. This observation is in accordance with a study by Xu et al.[22], who reported that EGCG suppresses the development of S. mutans biofilms due to its activity inhibiting glucosyltransferase, which reduces extracellular polysaccharide synthesis and bacterial adhesion. In line with this, Ferrazzano et al. demonstrated that green tea catechins decrease bacterial toxin production, thereby improving the anti-cariogenic potential.[7,23]

When compared to the total-etch technique, the self-etch green tea-incorporated adhesive showed a significantly greater zone of inhibition, proving that the adhesive protocol is crucial for enhanced antimicrobial efficacy. This result is in accordance with the research of Yu et al. [24], who showed that adhesives treated with EGCG had improved bonding stability in addition to its antibacterial action. Garcia-Contreras et al. [25] showed that EGCG-incorporated dental materials promoted odontoblast-like cell development in addition to its antimicrobial effects, supporting the multifunctional benefits of adding natural polyphenols to restorative materials.

In a systematic review of in vitro studies, Aragão et al. [26] consistently reported reduced viability, biofilm formation, and virulence factor production of S. mutans after EGCG treatment irrespective of variation in methodologies. The biological plausibility of the antimicrobial effects in this study is thus sustained. The systematic review and meta-analysis performed by Kiuru et al. [27] have further established the efficacy of inhibition of matrix metalloproteinases to improve resin-dentin bond

durability. While most studies in this meta-analysis have used chlorhexidine, a common pathway of inhibition highlights the interest of using natural compounds such as EGCG in dental adhesives.

The superior performances in the self-etching adhesive in this study can be linked to other procedural characteristics inherent in self-etching adhesives. The lack of a separate rinse step in self-etching adhesives can prevent losses of biologically active components absorbed into these materials. Van Meerbeeck et al. [28] observed in a study that self-etching adhesives leave a depot of hydroxyapatite surrounding a network of collagen fibers, which can be considered a reservoir for binding catechins in polyphenolic compounds. Such a characteristic can be attributed to the higher antioxidant and antimicrobial properties in self-etching adhesives.

Additionally, Landmayer et al. [29] demonstrated that EGCG treatment did not impair microtensile bond strength in eroded dentin, thus ensuring that EGCG did not affect adhesive penetration and/or polymerization in modified dentin. In a study concerning EGCG effects, Fialho et al. [30] assayed EGCG effects on nanoleakage in the adhesive–dentin interface in microscopically evaluated specimens and observed a marked decrease in EGCG-treated specimens, thus reinforcing EGCG's interfacial sealing effects in bonding dentin, which may be attributed to effects on dentin's endogenous enzymes. Sun et al. [31] observed increased thermal stability and hydrophobicity in EGCG-modified dentin collagen, ensuring increased resistance of the hybrid-layer zone to degradation, hence improved resistance to degradation in EGCG-modified dentin. Abreu et al. [32] studied EGCG-modified sound and eroded-abraded dentin bond strength, observing improved immediate and aged bond strength in sound and eroded-abraded dentin. Based on these studies, EGCG functions as a dentin biomodifier externally reinforcing bonding without impeding bonding performance, in accordance with elevated biologic responses in green tea-containing adhesive systems noted in this current study.

The current in vitro study is further strengthened by its holistic assessment of antioxidant and antimicrobial properties of green tea-incorporated dentin bonding agents using more than one analytical technique, and a head-to-head comparison of self-etch and total-etch adhesive approaches, thus making it more relevant in a practical setting. However, since this study took place in a controlled lab setting, it fails to totally imitate the complexities present in an oral microenvironment, with an assessment not being carried out for bond stability and polymerization characteristics in relation to green tea addition. The concentration used may not be directly clinically applicable, and future investigations should focus on optimizing lower, clinically feasible concentrations while simultaneously evaluating mechanical and bonding properties. Future studies should prioritize assessment of bond stability under both aging and thermomechanical loading, evaluation of chemical properties, and validate results using biofilm models.

CONCLUSION

Within the limitations of study, the incorporation of green tea extract into dentin bonding agents provided enhanced biological benefits without compromising adhesive bonding performance. The addition of green tea enhanced the antioxidant properties and showed antibacterial activity against Streptococcus mutans in both total-etch and self-etch adhesive systems, with the self-etch adhesive demonstrating consistently greater effect. The increased antibacterial activity and dose-dependent free radical scavenging capacity observed in self-etch adhesives indicate their superior ability to retain and express green tea polyphenols. Based on these findings the incorporation of green tea into dentin bonding agents holds a potential for the development of biologically active restorative materials with cariostatic abilities.

REFERENCES

1. Demarco FF, Corrêa MB, Cenci MS, Moraes RR, Opdam NJ. Longevity of posterior composite restorations: not only a matter of materials. Dent Mater. 2012;28(1):87–101.

2. Kidd EA. How 'clean' must a cavity be before restoration? Caries Res. 2004;38(3):305–13.

3. Spencer P, Ye Q, Park J, et al. Adhesive/dentin interface: the weak link in the composite restoration. Ann Biomed Eng. 2010;38(6):1989–2003.

4. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea—a review. J Am Coll Nutr. 2006;25(2):79–99.

5. Xu X, Zhou XD, Wu CD. Tea catechin epigallocatechin gallate inhibits Streptococcus mutans biofilm formation by suppressing gtf genes. Arch Oral Biol. 2012;57(6):678–83.

6. Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr. 2003;133(10):3275S–84S.

7. Ferrazzano GF, Amato I, Ingenito A, De Natale A, Pollio A. Anti-cariogenic effects of polyphenols from plant stimulant beverages. Fitoterapia. 2009;80(5):255–62.

8. Narotzki B, Levy Y, Aizenbud D, Reznick AZ. Green tea and its major polyphenol EGCG increase the activity of oral peroxidases. Arch Oral Biol. 2013;58(11):1630–5.

9. Epasinghe DJ, Yiu CKY, Burrow MF, et al. Effect of green tea extract on the durability of resin–dentin bonding. J Dent. 2015;43(6):743–52.

10. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater. 2011;27(1):17–28.
11. Faggion CM Jr. Guidelines for reporting pre-clinical in vitro studies on dental materials. Dent Mater. 2012;28(1):1–6.

12. Krithikadatta J, Gopikrishna V, Datta M. CRIS guidelines (Checklist for Reporting In-vitro Studies). J Conserv Dent. 2014;17(4):301–4.

13. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25–30.

14. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev. 1986;50(4):353–80.

15. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity. J Pharm Anal. 2016;6(2):71–9.
16. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically (M07). CLSI.
17. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate enhances the therapeutic potential of a dental adhesive. J Dent. 2012;40(6):485–492. doi:10.1016/j.jdent.2012.02.013.

18. Macedo FAA, Souza NO, Lemos MVS, et al. Dentin bonding and physicochemical properties of adhesives incorporated with epigallocatechin-3-gallate. Odontology. 2019;107(1):23–28. doi:10.1007/s10266-018-0367-0.

19. Yang H, Guo J, Deng D, Chen Z, Huang C. Effect of adjunctive application of epigallocatechin-3-gallate and ethanol-wet bonding on adhesive–dentin bonds. J Dent. 2016;44:44–49. doi:10.1016/j.jdent.2015.12.001.

20. Space for ur ref

21. Yu J, Zhang Z, Guo R, Peng W, Yang H, Huang C. Epigallocatechin-3-gallate/nanohydroxyapatite platform delivery approach to adhesive–dentin interface stability. Mater Sci Eng C Mater Biol Appl. 2021;122:111918. doi:10.1016/j.msec.2021.111918.

22. Xu X, Zhou XD, Wu CD. Tea catechin epigallocatechin gallate inhibits Streptococcus mutans biofilm formation by suppressing glucosyltransferase activity. Arch Oral Biol. 2012;57(6):678–683. doi:10.1016/j.archoralbio.2011.11.012.

23. **Space for ur ref**

24. Yu HH, Zhang L, Xu S, et al. Effects of epigallocatechin-3-gallate on the bonding stability of etch-and-rinse and self-etch adhesive systems. Materials (Basel). 2017;10(2):183. doi:10.3390/ma10020183.

25. Garcia-Contreras R, Chavez-Granados PA, Jurado CA, et al. Natural bioactive epigallocatechin-gallate promotes bond strength and differentiation of odontoblast-like cells. Biomimetics (Basel). 2023;8(1):75. doi:10.3390/biomimetics8010075.

26. Aragão MGB, Aires CP, Corona SAM. Effects of the green tea catechin epigallocatechin-3-gallate on Streptococcus mutans planktonic cultures and biofilms: systematic review of in vitro studies. Biofouling. 2022;38(7):687–695. doi:10.1080/08927014.2022.2116320.

27. Kiuru O, Sinervo J, Vähänikkilä H, Anttonen V, Tjäderhane L. MMP inhibitors and dentin bonding: systematic review and meta-analysis of immediate and aged bond strengths. Int J Dent. 2021;2021:9949699. doi:10.1155/2021/9949699.

28. Van Meerbeek B, Yoshihara K, Van Landuyt K, Yoshida Y, Peumans M. From Buonocore's pioneering acid-etch technique to self-adhering restoratives. J Adhes Dent. 2020;22(1):7–34. doi:10.3290/j.jad.a43994.

29. Landmayer K, Liberatti GA, Farias-Neto AM, Wang L, Honório HM, Francisconi-

Dos-Rios L. Effect of epigallocatechin-3-gallate on bond strength to eroded dentin. J Prosthet Dent. 2020;124(6):798.e1–798.e7. doi:10.1016/j.prosdent.2020.05.032.

30. Fialho MPN, Proença Nogueira F, Szesz AL, et al. Effect of epigallocatechin-3-gallate on nanoleakage and bond durability of adhesive interfaces. J Mech Behav Biomed Mater. 2019;91:398–405. doi:10.1016/j.jmbbm.2018.11.022.
31. Sun Q, Wang Y, Chen L, Zhang Z, Huang C. Epigallocatechin-3-gallate–mediated dentin biomodification: effects on collagen thermal stability and nanoleakage. *J Dent.* 2018;75:12–18.
32. Abreu BD, Almeida RF, Costa BB, Faria-e-Silva AL, Feitosa VP. Biomodification of sound and eroded-abraded dentin using epigallocatechin-3-gallate: effects on microtensile bond strength and interfacial characteristics. *J Adhes Dent.* 2023;25(4):345–352.

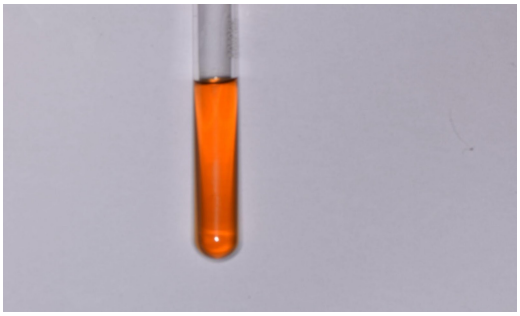

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Table 1: DPPH antioxidant activity (100 µL)

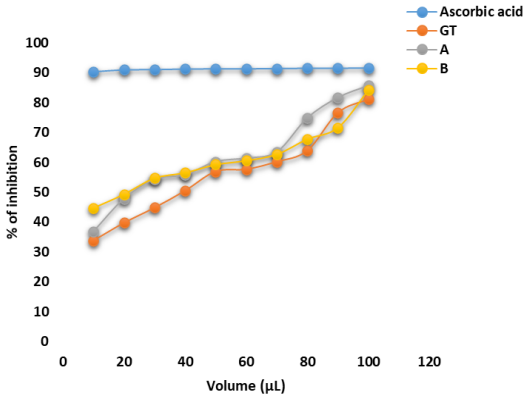

Figure 2: Dose-dependent DPPH radical scavenging activity (10–100 µL) of ascorbic acid, green tea extract, self-etch + green tea, and total-etch + green tea.

Sample	10 µL	20 µL	40 µL	60 µL	80 µL	100 µL	P value
Ascorbic acid	35.2 ± 2.1	50.1 ± 2.4	65.8 ± 2.0	75.6 ± 1.9	84.2 ± 2.1	91.6 ± 2.3	<0.001
Green tea extract	30.4 ± 2.0	45.6 ± 1.9	60.3 ± 2.1	68.9 ± 1.8	75.1 ± 2.0	81.7 ± 2.1	
Self-etch + green tea	25.8 ± 1.9	40.2 ± 2.0	55.1 ± 2.2	63.4 ± 1.7	70.3 ± 1.9	75.4 ± 2.0	
Total-etch + green tea	20.6 ± 1.8	35.4 ± 2.1	50.2 ± 2.0	58.1 ± 1.6	65.0 ± 1.7	68.2 ± 1.8	

Table 2: Dose-dependent DPPH activity

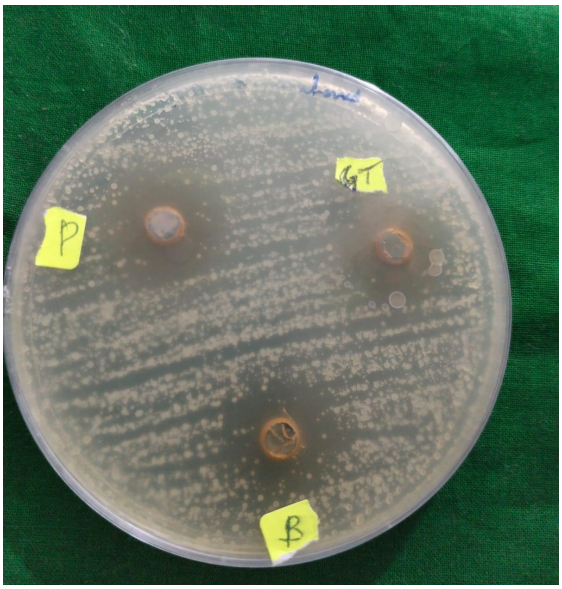

Figure 3: Antimicrobial activity (ZOI) against Streptococcus mutans