Effects of Plant Extracts on Dentin Bonding Strength: A Systematic Review and Meta-Analysis

Shikai Zhao†, Fang Hua2,3†, Jiarong Yan1, Hongye Yang1,4* and Cui Huang1,4*

1The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) and Key Laboratory for Oral Biomedical Ministry of Education, School and Hospital of Stomatology, Wuhan University, Wuhan, China, 2Department of Orthodontics, Center for Evidence-Based Stomatology, School and Hospital of Stomatology, Wuhan University, Wuhan, China, 3Division of Dentistry, School of Medical Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester, United Kingdom, 4Department of Prosthodontics, School and Hospital of Stomatology, Wuhan University, Wuhan, China

Objective: To systematically review in vitro studies that evaluated the effects of plant extracts on dentin bonding strength.

Materials and Methods: Six electronic databases (PubMed, Embase, VIP, CNKI, Wanfang and The Cochrane Library) were searched from inception to September 2021 in accordance with the Preferred Reporting Items for Systematic Reviews (PRISMA). in vitro studies that compared the performance of dental adhesives with and without the plant extracts participation were included. The reference lists of the included studies were manually searched. Two researchers carried out study screening, data extraction and risk of bias assessment, independently and in duplicate. Meta-analysis was conducted using Review Manager 5.3.

Results: A total of 62 studies were selected for full-text analysis. 25 articles used the plant extracts as primers, while five added the plant extracts into adhesives. The meta-analysis included 14 articles of in vitro studies investigating the effects of different plant extract primers on dentin bonding strength of etch-and-rinse and self-etch adhesives, respectively. The global analysis showed statistically significant difference between dental adhesives with and without plant extract primers. It showed that the immediate bond strength of dental adhesives was improved with the application of plant extract primers.

Conclusion: The application of proanthocyanidin (PA) primers have positive effect on the in vitro immediate bonding strength of dental adhesives irrespective of etch-and-rinse or self-etch modes.

Keywords: dentin, bonding, plant extracts, natural crosslinkers, adhesives, primers

INTRODUCTION

Dentin bonding is the foundation of esthetic restoration (Drummond, 2008). Nowadays, manufacturers claim that dental adhesive system has already developed to the eighth generation (Taneja et al., 2017). However, irrespective of acceptable immediate bonds, the long-term bonding strength of these adhesives is inadequate (Deligeorgi et al., 2001; Haas et al., 2016a). As a result, nearly half of esthetic restorations cannot serve for more than 10 years, and dentists have to spend 60% of...
working hours to replace them (Mjor et al., 2000; Deligeorgi et al., 2001). Thus, the improvement of long-term bond strength is still a puzzle that needs to be solved.

Unsatisfactory long-term dentin bonds are usually attributed to two reasons: The degradation of dentin collagen within the hybrid layer; and the emergence of secondary caries at the interface (Brackett et al., 2011). A reasonable strategy to solve these problems is to modify contemporary dental adhesives with different additives, such as chlorhexidine, nano-silver, carbon nanotube and amorphous calcium phosphate (Carrilho et al., 2007; Borges et al., 2013; Zhang et al., 2013; Alkatheeri et al., 2015). Amongst these additives, plant extracts attracted great attention due to their biological safety and functional versatility (Gotti et al., 2015; Yang et al., 2017; Yu et al., 2017). Many articles have reported the advantages of natural plant extracts, including their capability to stabilize dentin collagen (La et al., 2009), and to inhibit MMPs (Du et al., 2012; Yang et al., 2016) and microbes (Kaul et al., 1985; Rigano et al., 2007). Therefore, many researchers have been attempting to dope plant extracts into adhesives or provide a separate plant extract primer to achieve high antibiotic property and improved long-term bond strength (La et al., 2009; Borges et al., 2013; Gotti et al., 2015; Yang et al., 2017).

However, the combination of different adhesives with different plant extracts may produce unpredictable results, and different concentration of plant extract primer may have different bonding performance (Macedo et al., 2009; Islam et al., 2014). Previous studies have tested a limited amount of plant extracts, using different experimental designs, with contradictory conclusions. Thus, a comprehensive overview summarizing the effect of all existing plant extracts on dental adhesives will be helpful for dental clinicians and relevant researchers.

The objectives of this study are to systematically review the in vitro studies that evaluated adhesive-dentin bond strength with or without plant extracts participation and to compare different plant extracts in terms of bond strength. The hypotheses are: no difference exists in the bond strengths when modifying the adhesives with plant extracts; no difference exists in the bond strengths when plant extract primers are used; no difference exists in the bond strength when using different concentrations of plant extracts.

MATERIALS AND METHODS

Criteria for Considering Studies for This Review

Inclusion Criteria

- Studies that added plant extracts to dental adhesives or used plant extract as primers.
- Studies that compared the performance of dental adhesives with and without the participation of plant extracts.
- In vitro studies that evaluated the bond strength of dental adhesives.

Exclusion criteria

Reviews, clinical trials or case reports.

Search Strategy

A systematic electronic search was conducted by two independent reviewers (SZ and HY) using nine databases (PubMed, Embase, Web of Science, Cochrane Library, VIP, CNKI, Wanfang, OpenGrey literature and ProQuest Dissertation Abstracts) from inception to September 2021 to identify articles related to plant extracts and dental bonding. The search terms were a combination of subject terms and free-text terms (Appendix Table A1).

Data Collection and Analysis

Selection of Studies

Article titles and abstracts were independently screened by two authors (SZ and HY). The authors conducted a second review when the inclusion criteria were met. The abstracts were examined by two review authors (SZ and HY) independently using the same inclusion criteria. If there were disagreements, the abstract would be assessed by the third author (FH). Then full text of all potentially relevant studies were retrieved and independently assessed in duplicate by two review authors (SZ and HY). Any disagreement regarding the eligibility of the included studies was resolved through discussion with the third reviewer.

Data Extraction and Management

Data extraction was performed independently by two authors (SZ and HY). The demographic data, plant extracts used, plant extract concentration, bonding systems, as well as outcomes were recorded (Table 1). If any information was missing, we contacted the corresponding authors via email.

Quality Assessment

Two reviewers (SZ and HY) independently assessed the risk of bias of the included studies with the assessment instrument used in a previous systematic review of in vitro studies (Sarkis-Onofre et al., 2014). Quality assessment parameters included randomized teeth, teeth free of caries or restoration, operation following the manufacturer’s instructions, given sample size, and the bonding procedures were performed by a single operator with or without blinding. The article would be given a “Yes” on the parameter if it was reported and performed appropriately in the article; and a “No” if it was not mentioned or not performed properly. Articles were classified into three levels of risk of bias according to the number of parameters that scored “Yes”: high (≤2 parameters), medium (3-4 parameters), and low (5-6 parameters) (Table 2).

Statistical Analysis

Meta-analysis was conducted using Review Manager 5.3. Each possible comparison of the bond strength of dental adhesives with or without plant extracts participation was undertaken. In order to minimize the heterogeneity, only in vitro studies comparing the same plant extracts with the same concentration was included.
TABLE 1 | Characteristics of the included studies.

| First author          | Year | Country | Publication                  | Plant extracts | Action modes | Plant extracts concentration | Dental adhesives            | Outcome              |
|-----------------------|------|---------|------------------------------|----------------|--------------|------------------------------|-----------------------------|----------------------|
| Albuquerque N         | 2019 | Brazil  | Oper Dent                    | EGCG           | Adhesive     | 0.1% w/v                    | Single Bond 2 (3M ESPE, St. Paul, MN, United States) | MTBS                 |
| Yang H                | 2017 | China   | SCI REP                      | Quercetin      | Adhesive     | 100, 500, and 1,000 μg/ml   | Single Bond 2              | MTBS                 |
| Yu HH                 | 2017 | China   | Materials (Base)             | EGCG, EGCG-3Me | Adhesive     | 200, 400, and 600 μg/ml     | Single Bond 2              | MTBS                 |
| Gotti VB              | 2015 | Brazil  | J Adhes Dent                 | Quercetin      | Adhesive     | 5 wt%                       | Single Bond 2; Clearfil SE Bond (Kuray Noritake Dental; Tokyo, Japan); Easy Bond (3M ESPE, St. Paul, MN, United States) | MTBS                 |
| Du X                  | 2012 | China   | J Dent Materials             | EGCG           | Resveratrol  | 100, 200, and 300 μg/ml     | Single Bond 2              | MTBS                 |
| Peng W                | 2020 | China   | Materials and Engineering C  | EGCG           | Primer       | 0.01%, 0.1%, 1%             | Single Bond Universal      | MTBS                 |
| Zhang Z               | 2020 | China   | J Adhes Dent                 | EGCG           | Primer       | EGCG at 400 μM, 10% PA      | Single Bond Universal      | MTBS                 |
| Landmayer K           | 2020 | Brazil  | J Prosthet Dent              | EGCG; Proanthocyanidin (PA) | Primer       | 0.065                       | Single Bond Universal      | MTBS                 |
| Dávila-Sánchez A      | 2020 | Chile   | Dent Mater                   | EGCG           | Primer       | 0.065                       | Prime and Bond Elect (Dentsply Strona, Milford, DE, United States); Single Bond Universal; Tetric n-Bond Universal (Ivoclar Vivadent AG, Schaan, Liechtenstein) | MTBS |
| de Siqueira FSF       | 2020 | Brazil  | Clin Oral Investig           | Baicalein      | Primer       | 0.01%, 0.05%, and 1% w/v    | Single Bond Universal      | MTBS                 |
| Yi L                  | 2019 | China   | J Dent                       | Baicalein      | Primer       | 0.1% EGCG; or 1% PLGA/EGCG  | Single Bond 2              | MTBS                 |
| Albuquerque N         | 2019 | Brazil  | Oper Dent                    | EGCG           | Primer       | 0.05                        | Single Bond Universal      | MTBS                 |
| Costa CAG             | 2019 | Brazil  | J Adhes Dent                 | EGCG           | Primer       | 0.2%, 0.2%; 0.5%            | Single Bond 2              | MTBS                 |
| Fialho MPN            | 2019 | Brazil  | J Mech Behav Biomed Mater    | EGCG           | Primer       | 0.02%                       | Single Bond Universal      | MTBS                 |
| Li J                  | 2018 | China   | Oper Dent                    | Baicalein      | Primer       | 0.1, 0.5, 2.5, and 5.0 μg/ml| Single Bond 2              | MTBS                 |
| Porto ICCM            | 2018 | Brazil  | Eur J Oral Sci               | Baicalein      | Primer       | 100, 250, 500, or 1,000 μg/ml| Single Bond 2              | MTBS                 |
| Bacelar-Sá R          | 2017 | Brazil  | Braz Dent J                  | Proanthocyanidin| Primer       | 0.065                       | Single Bond Universal; Prime and Bond Elect; All-Bond 3 (Bisco Inc., Schaumburg, IL, United States); G-Aerial (GC Corp., Tokyo, Japan) | MTBS |
| Li K                  | 2017 | China   | RSC Adv                      | Quercetin      | Primer       | 0.01, 0.5, and 0.5 mass%    | Single Bond 2              | MTBS                 |
| Zheng P               | 2017 | China   | Sci Rep                      | Proanthocyanidin| Primer      | 0.06                        | Single Bond 2              | MTBS                 |
| Zhou J                | 2016 | China   | Dent Mater                   | Grape seed extract | Primer | 6.5 wt%                     | Single Bond Plus; Tetric N-Bond | MTBS |
| Hass V                | 2016 | Brazil  | Dent Mater                   | Proanthocyanidin| Primer | 0.02% and 0.1%              | Single Bond 2              | MTBS                 |
| Yang H                | 2016 | United States | J Dent | EGCG | Primer | 0.0005                     | Single Bond 2              | MTBS                 |
| Zheng P               | 2015 | United States | Oper Dent | Grape seed extract | Primer | 0.5%, 1%, 2%, 5% of hesperidin (HPN) or 0.5% of proanthocyanidins (PA) | OptiBond FL (Kerr, Scafati, Italy); Clearfil SE Bond | MTBS |
| Islam MS              | 2014 | Japan   | Dent Mater                   | Proanthocyanidin; Hesperidin | Primer | 0.005                      | Clearfil SE Bond             | MTBS |
| Liu RR                | 2014 | China   | Int J Oral Sci               | Proanthocyanidin| Primer | 10% or 15%                  | Single Bond 2              | MTBS                 |
| Santiago SL           | 2013 | Brazil  | J Adhes Dent                 | Proanthocyanidin| Primer | 0.02%, 0.1%, or 0.5% w/v   | Single Bond 2              | MTBS                 |
| Broyles AC            | 2013 | United States | J Prosthodont | Grape seed extract | Primer | 0.065                      | Single Bond 2              | MTBS                 |

(Continued on following page)
The mean difference with 95% confidence interval (CI) was calculated and \( p \leq 0.05 \) was considered significant. Statistical heterogeneity was assessed using the modified chi-square test (Cochran's Q), which indicates heterogeneity when \( p > 0.1 \), and \( I^2 \) test, which indicates heterogeneity when its values is greater than 50%. Random-effect model was used in the analysis. The publication bias was to be assessed if more than ten studies were included in a meta-analysis. Sensitivity analysis was also performed by sequentially excluding each study if there were sufficient studies (\( \geq 10 \)).

### RESULTS

**Search Strategy and Characteristics**

The initial search yielded 341 articles, out of which, 36 articles were eliminated after screening of titles and removal of duplicates. After abstract screening, 243 articles were excluded. A resultant sample of 62 articles was carried forward to the next stage, in which full-text copies were scrutinized. Finally, a total of 30 studies were systematically reviewed, in which 5 studies added plant extracts into adhesives and 25 studies used plant extract solution as primers (Figure 1). Twenty-nine articles were in

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**TABLE 1** | Characteristics of the included studies.

| First author | Year | Country | Publication | Plant extracts | Action modes | Plant extracts concentration | Dental adhesives | Outcome |
|--------------|------|---------|-------------|---------------|-------------|-----------------------------|------------------|---------|
| Liu RR       | 2012 | China   | Zhonghua Kou Qiang Yi Xue Za Zhi | Proanthocyanidin | Primer | 0.15 | Single Bond 2 | MTBS |
| Macedo GV    | 2009 | United States | J Dent Res | Grape seed extract | Primer | 0.065 | Single Bond 2; One Step Plus (Bisco, Schaumburg, IL, United States) | MTBS |
| Al-Ammar A   | 2009 | United States | J Biomed Mater Res B Appl Biomater | Grape seed extract; Genipin | Primer | 6.5% GSE; 0.5% GE | One Step Plus; Single Bond Plus | MTBS |

Abbreviation: EGCG, epigallocatechin-3-gallate; EGCG-3Me, epigallocatechin-3-O-(3-O-methyl)-gallate; GSE, grape seed extract; GE, genipin.

| Study | Year | Random | Caries | Adhesive | Sample | Operator | Blind | Risk |
|-------|------|--------|--------|----------|--------|----------|-------|------|
| Peng W | 2020 | Y      | Y      | Y        | Y      | Y        | Y     | N    | Low |
| Zhang Z | 2020 | Y      | N      | Y        | Y      | Y        | Y     | N    | Medium |
| Landmayer K | 2020 | N      | Y      | Y        | Y      | N        | Y     | N    | Medium |
| Dávila-Sánchez A | 2020 | Y      | Y      | Y        | Y      | Y        | Y     | N    | Low |
| de Souza FSF | 2020 | Y      | Y      | Y        | Y      | Y        | Y     | N    | Low |
| Albuquerque N | 2019 | Y      | Y      | Y        | N      | Y        | N     | N    | Medium |
| Yi L | 2019 | Y      | Y      | Y        | Y      | N        | Y     | N    | Low |
| Albuquerque N | 2019 | N      | Y      | Y        | Y      | N        | Y     | N    | Medium |
| Costa CAG | 2019 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Fialho MPN | 2019 | N      | Y      | Y        | Y      | Y        | N     | N    | Medium |
| Li J | 2018 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Porto JCOM | 2018 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Yang H | 2017 | Y      | Y      | Y        | Y      | N        | Y     | N    | Medium |
| Yu HH | 2017 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Bacelar-Sá R | 2017 | N      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Li K | 2017 | Y      | Y      | Y        | N      | Y        | N     | N    | Medium |
| Zheng P | 2017 | N      | Y      | Y        | N      | Y        | N     | N    | Medium |
| Zhou J | 2016 | N      | Y      | Y        | N      | Y        | N     | N    | Medium |
| Hass V | 2016 | N      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Yang H | 2016 | N      | Y      | Y        | Y      | Y        | N     | N    | Medium |
| Gotti VB | 2015 | Y      | N      | Y        | Y      | Y        | N     | N    | Medium |
| Zheng P | 2015 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Islam MS | 2014 | N      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Liu RR | 2014 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Santiago SL | 2013 | N      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Broyes AC | 2013 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Du X | 2012 | Y      | N      | Y        | Y      | N        | N     | N    | Medium |
| Liu RR | 2012 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Macedo GV | 2009 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
| Al-Ammar A | 2009 | Y      | Y      | Y        | Y      | N        | N     | N    | Medium |
English and 1 were in Chinese. There are nine types of plant extracts and 15 types of adhesives involved (Table 1).

**Risk of Bias**
Most of the 30 studies (86.7%) exhibited a medium risk of bias, except for four (13.3%) with a low risk of bias. All of the studies used the adhesive according to the manufacturer’s instructions and described sample size calculation, but none of the studies reported blinding. A total of 20 studies (66.7%) reported random assignment of teeth, and 27 studies (90%) used teeth free of caries. Only seven studies (23.3%) reported adhesive procedure performed by a single operator. The results are described in Figure 2 and Table 2.

**Meta-Analysis**
In the studies included in the meta-analysis, we only choose the data of interest. Only commercial adhesives were included, and the studies used experimental adhesives were excluded (Epasinghe et al., 2012). The effect of plant extracts on bonding strength may be related to different bonding modes such as self-etch or etch-and-rinse (Macedo et al., 2009; Bacelar-Sa et al., 2017). Hence, the disparity of the bond strength of different plant extracts in self-etch or etch-and-rinse adhesives was compared. Because aging methods were highly heterogeneous (i.e., water storage, saliva storage and PH cycling), it was not considered in the meta-analysis (Deng et al., 2014).

Due to the fact that different concentrations of plant extracts were used, only those with the same concentration were taken into meta-analysis. Of the 30 studies, data from 14 papers in which plant extract solution serve as primers underwent meta-analysis. The results of the meta-analysis are shown in Figures 3–5.

**Etch-and-Rinse Bond Strength (Plant Extract Primers)**
The first analysis (etch-and rinse adhesive with or without plant extract primers) was performed, and the different concentration of plant extracts were the subgroups. A total of 29 datasets were selected, while 14 studies were included (Figure 3), with the following results: Q-test $p < 0.00001$, $I^2 = 95\%$ and overall effect $p = 0.0007$. Test for subgroup differences: Q-test $p = 0.02$ and $I^2 = 69.7\%$, which showed that the data of subgroups were consistent.

The data of subgroup using 0.1% EGCG as primer showed no statistically significant differences compared with control group (Z-test: $p > 0.05$). However, the result of proanthocyanidin (PA) showed that the experimental groups had significant higher bond strengths than the control groups, with overall effect $p < 0.05$. For primers with 5% PA and 6.5% PA, the result in the Q-test was both $p < 0.01$ and $I^2 = 98\%$, $I^2 = 91\%$, separately. However, the result of 10% PA in the Q-test was $p > 0.05$ and $I^2 = 0\%$. The results of the meta-analysis are shown in Figure 3.

**Self-Etch Bond Strength (Plant Extract Primers)**
For the second analysis (self-etch adhesive with or without plant extract primers), 10 data sets were selected, with four studies.
FIGURE 2 | Risk of bias graph judgements about each risk of bias item presented as percentages across all included studies.

FIGURE 3 | Forest Plot—plant extract primers: etch-and-rinse immediate bond strength,
FIGURE 4 | Forest Plot—plant extract primers: self-etch immediate bond strength.

FIGURE 5 | Forest Plot—proanthocyanidin (PA) primers

| Study or Subgroup | PA Control | Mean Difference | Mean Difference |
|-------------------|------------|-----------------|----------------|
|                    |            | IV Random 95% CI | IV Random 95% CI |
| Baceteria-SA R 2017 - PAGA | 20.7 9.8 | 5 12.2 7 5 6.4% 6.90 [1.36, 18.36] |
| Baceteria-SA R 2017 - PASBU | 64.7 9.1 | 5 55.2 5.5 5 6.8% 9.50 [0.18, 18.82] |
| Broyles AC 2013 - GSE G | 8.3 4.3 | 5 11.4 6.5 5 10.2% -3.10 [-9.93, 3.73] |
| Broyles AC 2013 - GSE U | 13 7.6 | 5 12.2 5.1 5 8.5% 0.80 [-7.22, 8.82] |
| de Siqueira FSF 2020 - PASBU| 29.3 3.8 | 7 20.1 4.4 7 15.4% 9.20 [4.89, 13.51] |
| de Siqueira FSF 2020 - PACEU| 44.5 5.4 | 7 31 4.3 7 13.5% 13.50 [8.39, 18.61] |
| de Siqueira FSF 2020 - PATEU| 28.7 3.6 | 7 20.7 2.6 7 17.6% 8.00 [4.82, 11.18] |
| de Siqueira FSF 2020 - PATEUS| 37.4 3.9 | 7 30.6 2.6 7 17.4% 6.80 [3.33, 10.27] |
| Macedo GV 2009 - GSEOS| 73.14 17 | 6 65.22 20.4 6 1.8% 7.92 [-13.33, 29.17] |
| Macedo GV 2009 - GSEOSP| 53.9 4 | 22.2 | 5.3 3.8 4 | 3.3% 0.20 [-4.65, 5.05] |

Subtotal (95% CI) 27 29 23.1% 14.19 [0.48, 27.90]

Heterogeneity: $I^2 = 93.8$; $H^2 = 100.00$, df = 9 ($P < 0.00001$); $P = 93$

Test for overall effect: $Z = 4.86$ ($P = 0.00001$)

| Study or Subgroup | PA Control | Mean Difference | Mean Difference |
|-------------------|------------|-----------------|----------------|
|                    |            | IV Random 95% CI | IV Random 95% CI |
| Al-Ammar A 2009 - GSEOS| 74.4 10.8 | 6 44.3 8.5 8 3.0% 30.27 [21.12, 39.42] |
| Al-Ammar A 2009 - GSESB| 71.06 14.9 | 8 33.8 8.7 8 2.8% 37.68 [26.53, 48.83] |
| Baceteria-SA R 2017 - PAAB | 63.8 4.4 | 5 48.1 10.2 5 3.0% 15.70 [9.56, 21.84] |
| Baceteria-SA R 2017 - PAPBE | 63 13.4 | 5 51.5 6.3 5 2.7% 11.10 [8.88, 24.08] |
| de Siqueira FSF 2020 - PAPBES | 23.7 3.1 | 7 16.5 3.6 7 3.4% 7.20 [6.10, 10.72] |
| de Siqueira FSF 2020 - PAPBESf | 28.2 4 | 7 23.5 3.4 7 3.4% 4.70 [0.61, 8.59] |
| Oliva-Sanchez A 2020 - PA | 20.66 3.9 | 7 14.2 4.3 7 3.4% 6.24 [4.06, 10.62] |
| Hass V 2016 - PASB | 36.2 | 5.5 | 5 39.7 7.9 | 5 3.1% -3.90 [-11.94, 4.94] |
| Hass V 2016 - PATN | 29.2 1.2 | 5 36.8 4.7 | 5 3.4% -7.60 [-11.85, -3.35] |
| Macedo GV 2009 - GSESB | 68.34 23.8 | 66 59.62 20 | 6 3.7% 8.72 [16.15, 33.59] |
| Macedo GV 2009 - GSESB ED | 55.9 14 | 6 36.75 8.5 | 6 2.7% 19.15 [0.04, 32.26] |

Subtotal (95% CI) 69 69 32.4% 11.05 [4.39, 17.72]

Heterogeneity: $I^2 = 102.58$; $H^2 = 111.23$, df = 10 ($P < 0.00001$); $P = 91$

Test for overall effect: $Z = 3.25$ ($P = 0.001$)

| Study or Subgroup | PA Control | Mean Difference | Mean Difference |
|-------------------|------------|-----------------|----------------|
|                    |            | IV Random 95% CI | IV Random 95% CI |
| Baceteria-SA R 2017 - PAGA | 20.7 9.8 | 5 12.2 7 5 3.0% 0.50 [-1.36, 18.36] |
| Baceteria-SA R 2017 - PASBU | 64.7 9.1 | 5 55.2 5.5 5 3.0% 9.50 [0.18, 18.82] |
| Broyles AC 2013 - GSE G | 8.3 4.3 | 5 11.4 6.5 5 3.2% -3.10 [-9.93, 3.73] |
| Broyles AC 2013 - GSE U | 13 7.6 | 5 12.2 5.1 5 3.0% 0.80 [-7.22, 8.82] |
| de Siqueira FSF 2020 - PASBU| 29.3 3.8 | 7 20.1 4.4 7 3.4% 9.20 [4.89, 13.51] |
| de Siqueira FSF 2020 - PACEU| 44.5 5.4 | 7 31 4.3 7 3.3% 13.50 [8.39, 18.61] |
| de Siqueira FSF 2020 - PATEU| 28.7 3.6 | 7 20.7 2.6 7 3.4% 8.00 [4.62, 11.18] |
| de Siqueira FSF 2020 - PATEUS| 37.4 3.9 | 7 30.6 2.6 7 3.4% 6.80 [3.33, 10.27] |
| Macedo GV 2009 - GSEOS| 73.14 17 | 6 65.22 20.4 6 1.9% 7.92 [-13.33, 29.17] |
| Macedo GV 2009 - GSEOSP| 52.85 18.2 | 6 37.38 14.8 | 6 2.1% 15.47 [-3.30, 34.24] |

Subtotal (95% CI) 60 60 29.8% 7.27 [4.34, 10.21]

Heterogeneity: $I^2 = 9.75$; $H^2 = 18.95$, df = 9 ($P = 0.03$); $P = 53$

Test for overall effect: $Z = 4.66$ ($P < 0.00001$)

Total (95% CI) 175 177 100.0% 9.60 [5.22, 13.97]

Heterogeneity: $I^2 = 143.00$; $H^2 = 56.45$, df = 32 ($P < 0.00001$); $P = 99$

Test for overall effect: $Z = 4.30$ ($P < 0.00001$)

Test for subgroup differences: $H^2 = 3.72$; $df = 3$ ($P = 0.29$); $P = 19.4$

FIGURE 5 | Forest Plot—proanthocyanidin (PA) primers
included (Figure 4). The results were as follows: Q-test \( p < 0.05 \) and \( I^2 = 53\% \). The global analysis showed statistically significant difference \( p < 0.05 \).

**Primers With Vs Without Proanthocyanidin**

For the third analysis (primers with or without PA), 11 studies and 33 datasets were included (Figure 5). The difference between control and experimental groups were statistically significant (Q-test: \( p < 0.01 \), \( I^2 = 95\% \) and Z-test: \( p < 0.05 \)). The differences in the test for subgroups (primers with different concentration of PA) showed the following values: chi-squared = 3.72, df = 3 \( (p = 0.29) \) and \( I^2 = 19.4\% \). The meta-analysis results are shown in Figure 5.

**DISCUSSION**

This systematic review is the first to verify the effects of plant extracts on dentin bonding strength from *in vitro* studies. Thorough database research was conducted, and data were extracted and integrated in tables. Each study was designed and performed on the basis of their own parameters (plant extract types, action modes, concentration, dental adhesives and indicators), as listed in Table 1. Nine different plant extracts were added into 15 types of adhesives or served as primers. Out of the 30 studies, the data from 14 were selected for further evaluation.

As shown in Table 1, there were different commercial adhesives used. We had undertaken several measures to avoid the discrepancy. Firstly, the details of the adhesives were listed, such as commercial name, manufacturer, and place of production. Secondly, the articles that used experimental adhesives were excluded in the present study. Thirdly, 19 of 30 included studies chose the same one commercial adhesive, Single Bond 2 (3M ESPE, St. Paul, MN, United States). Furthermore, all included studies set the control group which did not add plant extracts into adhesives or serve as primers. All these strategies were helpful to eliminate the disturbance of different adhesives on research results to the utmost extent. Furthermore, the included studies all reported the manufacturers and details of plant extracts, such as resveratrol powder (Sigma–Aldrich, St. Louis, MO, United States), and the pureness of this product was listed as \( \geq 99\% \) (HPLC).

Since plant extract was introduced, its effectiveness in crosslinking and biocompatibility has drawn a lot of attention. Different plant extracts were investigated, listing as follows: proanthocyanidin (PA), epigallocatechin-3-gallate (EGCG), quercetin (QUE), resveratrol (RSV), baicalein (BAI), hesperidin (HES), rutin (RUT) and naringin (NAR). Firstly, despite the chemical structure differences, they all belong to plant polyphenol, which possesses antioxidant and anti-inflammatory properties. These effects are mainly derived from phenolic hydroxyl groups in polyphenols (Leopoldini et al., 2011). The highly-hydroxylated structures make them capable of forming insoluble complexes with carbohydrates and proteins (Bravo, 1998; Teixeira et al., 2002). The major force that stabilizes the plant-extract-protein complexes is hydrogen bonding between phenolic hydroxyl and peptide carbonyl (Hagerman and Butler, 1980a; Hagerman and Butler, 1980b), which is strengthened by alkyl substitution on the amide nitrogen adjacent to the carbonyl (Cannon, 1955). Therefore, the mechanical properties of collagen complex would be increased (Yang et al., 2016). Secondly, plant extracts, such as baicalein and resveratrol, can inhibit the activity of peptidases directly or indirectly by changing the catalytic domain (Mazzoni et al., 2018) or crosslinking with noncollagenous proteins regulating peptidases (Breschi et al., 2010; Cova et al., 2011). Thirdly, many plant extracts, like baicalein, are commonly used in herbal medicines to treat bacterial and viral infections. They show remarkable antimicrobial effects on different bacteria including *Escherichia coli*, *P. cuspidatum* and *S. mutans* (Song et al., 2006; Duan et al., 2007; Zeng et al., 2008; Chinnam et al., 2010; Jang et al., 2014). The mechanisms are not clear yet, but it might be attributed to the inhibition of the cellular growth (Paulo et al., 2010).

One of the most studied plant extracts is proanthocyanidin (PA), also known as grape seed extracts (GSE) (Al-Ammar et al., 2009; Macedo et al., 2009; Liu et al., 2012; Broyles et al., 2013; Liu et al., 2014; Islam et al., 2014; Zheng et al., 2015; Zhou et al., 2016; Hass et al., 2016b; Bacellar-Sa et al., 2017; Zheng and Chen, 2017; de Siqueira et al., 2020; Ds et al., 2020; Landmayer et al., 2020). It is a condensed tannins extracted from *Vitis vinifera* grapes, which has been reported to contain 79.6% polyphenols (Aguiar et al., 2014). PA is composed of flavon-3-ol subunits, catechin, epicatechin and epicatechin-3-O-gallate and linked through C4-C8 (Cavaliere et al., 2010). These components are responsible for their properties such as free-radical scavenging capacity, high affinity for protein, antioxidant potential and capacity to enhance the mechanical properties of collagen (Castellan et al., 2010; Leme-Kraus et al., 2017). Epasinghe et al. (2012) reported that incorporation of less than 3% proanthocyanidin into dental adhesive can reduce nanoleakage without comprising 24 h adhesive-dentin bond strength. The meta-analysis of the PA primer effects on bonding showed a significant positive effect compared with the control group, irrespective of the concentrations or the type of adhesive used (Al-Ammar et al., 2010; Macedo et al., 2009; Liu et al., 2014; Wiegand et al., 2015; Zhou et al., 2016; Hass et al., 2016a; Bacellar-Sa et al., 2017; Ds et al., 2020; Landmayer et al., 2020; Siqueira et al., 2020). However, the results of 5 and 6.5% PA primer revealed a heterogeneity of 98% and 91% (Figure 3). The reason might be attributed to different bonding techniques such as dry bonding and wet bonding (Zhou et al., 2016) For 10% PA primer, the bonding strength shows statistically significant elevation with no heterogeneity (Liu et al., 2014; Landmayer et al., 2020). Although the heterogeneity varies from group to group, the subgroup analysis revealed no significant differences, which also prove the effectiveness of PA primer.

Another important plant extract being intensely investigated is epigallocatechin-3-gallate (EGCG) (Du et al., 2012; Santiago et al., 2013; Yang et al., 2016; Yu et al., 2017; Albuquerque et al., 2019; Costa et al., 2019; Fialho et al., 2019; Landmayer et al., 2020; Zhang et al., 2020). It is one of the flavanols in tea, also known as catechins (Tachibana, 2011). As a representative component of green tea, it cannot be found in any plants except *C. sinensis* (L.) Kuntze (Tachibana, 2011). EGCG consists of a meta-5,7-dihydroxy-substituted A ring and trihydroxy phenol structures.
on both the B and D rings (Peter et al., 2017). The polyphenolic structure makes EGCG good donors for hydrogen bonding (Yang et al., 2009). Thus, it has shown the ability to bring various health benefits, like anti-metastasis, anti-inflammatory and antioxidant effects (Mukhtar and Ahmad, 2000; Mereles and Hunstein, 2011; Suzuki and Isemura, 2013). The similarity of chemical structure with other flavanols like PA makes it capable of enhancing the mechanical strength of collagen. The addition of EGCG directly into adhesives has been proven to preserve the bond strength after different ageing methods (Du et al., 2012; Yu et al., 2017; Albuquerque et al., 2019). The result of EGCG primer showed no negative influence on immediate bond strength (Zhang et al., 2020). The lack of data and various ageing methods make it impossible to do meta-analysis on aged bond strength. However, plenty of articles showed EGCG primer can improve the bond stability (Landmayer et al., 2020; Zhang et al., 2020). Furthermore, Yu et al. (2017) created a derivative of EGCG, called EGCG-3Me, which can enhance the bond stability, inhibited S.mutans adhesion and hinder its growth.

The plant extracts are normally recognized as plant polyphenols, which encompass a wide variety of molecules that contain at least one aromatic ring with one or more hydroxyl groups (Ferrazzano et al., 2011). Although they were extracted from different plants, the similarity in their chemical structure makes it possible for them to all possess properties like antioxidation and anti-bacterium. To begin with, the highly-hydroxylated structures make them capable of forming complexes with proteins, especially proline-rich proteins in dental collagen (Bravo, 1998). This fortiﬁed crosslinking interaction helps enhance the mechanical strength of dental bonding (Yang et al., 2017; Yi et al., 2019; Peng et al., 2020). Furthermore, the polyphenolic compounds could coordinate with metal ions and compete with peptidases such as MMPs for the catalytic domain in collagen (Mazzoni et al., 2018). As a

| Plant extracts | Molecular formula | Mol. Weight (g/mol) | Number of hydroxyphenyl radicals | Effects on immediate bonding strength |
|----------------|-------------------|---------------------|----------------------------------|-------------------------------------|
| Proanthocyanidin | C26H26O13 | 594.5 | 7 | Green |
| Epigallocatechin gallate | C22H18O11 | 458.4 | 8 | Green |
| Quercetin | C15H10O7 | 302.2 | 5 | Green |
| Resveratrol | C14H12O3 | 228.2 | 3 | Green |
| Baicalein | C15H10O5 | 270.2 | 3 | Green |
| Genipin | C17H14O6 | 226.2 | 2 | Green |
| Hesperidin | C18H14O15 | 610.6 | 2 | Green |
| Rutin | C20H16O16 | 610.5 | 4 | Green |
| Naringin | C20H12O14 | 580.5 | 2 | Green |

Green = evident; Yellow = unclear; Red = not recommended.
result, the enzymatic hydrolysis of hybrid layer collagen would be impeded and the adhesive-dentin interface stability would be maintained (Epasinghe et al., 2012; Yang et al., 2016; Yang et al., 2017). Besides, the plant polyphenols were considered metabolites involved in the chemical defense of plants and possess the ability to inhibit bacteria (Ferrazzano et al., 2011). There is plenty of evidence supporting the inhibition of cariogenic bacteria by phenolic compounds. The mechanisms of polyphenols against bacteria like S. mutans may include affecting cell membrane permeability, inhibiting protein synthesis, blocking ATP synthesis and inhibiting bacterial metabolism (Chinnam et al., 2010; Xie et al., 2015). Lastly, as natural crosslinkers, the plant polyphenols are non-toxic compared to synthetic compounds like chlorhexidine and glutaraldehyde. They can protect cells by inhibiting oxidative stress-induced DNA damage, lipid peroxidation and protein oxidation (Kang et al., 2012). To conclude, all these in vitro studies demonstrated that the plant extracts, consisting of polyphenols, can enhance mechanical strength of dentin collagen, maintain dentin-adhesive stability, inhibit cariogenic bacteria and resist adhesive-induced cytotoxicity.

Although plant extracts have shown plenty of advantages, there are still a large variety of aspects to be explored, such as solvent, treatment time and concentrations. First, theoretically, the effect of plant extracts would increase with the concentration. However, the solubility of the compounds were not great (Bravo, 1998). Zhang et al. (2020) reported EGCG with dimethyl sulfoxide as a solvent can exert synergistic effect on dentin-adhesive interface stability. Second, the treatment time varies from one to another. Genipin is reported to have a slow rate of cross-linking induction that the mechanical strength increased only after 40 h treatment (Bedran-Russo et al., 2007). Third, the effect of different concentration on bonding is complex. It has been shown more than 3% PA added into adhesive directly can exert adverse effect on bonding (Epasinghe et al., 2012). More studies are needed to determine the suitable solvent, treatment time and concentrations of plant extracts.

As mentioned in this review, plant extracts are actually polyphenols, which possess phenolic hydroxyl groups and aromatic rings (Ferrazzano et al., 2011). Therefore, their solubility in solvents such as ethanol are high, due to their similar chemical structure like hydroxyl groups. Furthermore, the interactions between plant extract (eg. PA) and collagen can be disrupted by detergents of hydrogen bond-weakening solvents, suggesting that PA-collagen complex formation involves primarily hydrogen bonding between the protein amide carbonyl and the phenolic hydroxyl (Hagerman and Klucher, 1986). Ethanol, on the other hand, stimulate PA and collagen interactions (Bo et al., 2010). There is no evidence that the interaction is concentration-dependent.

The present study showed the changes in dentin bond strength after adding plant extracts into adhesives or serving as primers. Although strict selection was performed to minimize heterogeneity, the data of several subgroups remained high heterogeneous. There are three reasons for heterogeneity: 1. Different adhesive brands; 2. Different bonding modes (etch-and-rinse or self-etch); 3. Different dentin material (normal or eroded dentin). Also, several authors failed to report important details, such as whether the same operator performed the bonding steps of all specimens. These factors may help explain the high heterogeneity in in vitro experiments.

### CONCLUSIONS

Plant extracts have positive effects on the immediate microtensile bond strength of the adhesive-dentin interface. Meta-analysis demonstrated that the use of proanthocyanidin (PA) primer, especially at the concentration of 10%, had statistically significant effect on the immediate dentin bonding strength. Considerable heterogeneity existed among the different adhesive brands, bonding modes and dentin materials used, which limited the meta-analysis approach. Further clinical research is needed to confirm the effect of plant extracts on bond strength in vivo.

### DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

### AUTHOR CONTRIBUTIONS

The conception and design of the study were performed by HY and CH. Literature retrieving and studies selection were performed by SZ and JY. Quality evaluation was carried out by CH and JY. Mathematical modeling and meta-analysis were conducted by FH and SZ. Results analysis and interpretation were undertaken by HY and SZ. The manuscript was drafted by HY and SZ. All authors read and approved the final manuscript.

### FUNDING

This work was financially supported by National Natural Science Foundation of China (81701012), Youth Clinical Research Fund of Chinese Stomatological Association (CSA-B2018-01).

### REFERENCES

Aguir, T. R., Vidal, C. M. P., Phansalkar, R. S., Todorova, I., Napolitano, J. G., Mcalpine, J. B., et al. (2014). Dentin Biomodification Potential Depends on Polyphenol Source. *J. Dent. Res.* 93 (4), 417–422. doi:10.1177/0022034514523783

Al-Ammar, A., Drummond, J. L., and Bedran-Russo, A. K. (2010). The Use of Collagen Cross-Linking Agents to Enhance Dentin Bond Strength. *J. Biomed. Mater. Res. B Appl. Biomater.* 91 (1), 419–424. doi:10.1002/jbm.b.31417
Bo, H., Jaurequi, J., Bao, W. T., and Nimni, M. E. (2010). Proanthocyanidin: a
natural Crosslinking Reagent for Stabilizing Collagen Matrices. J. Biomed.
Mater. Res. A 65A.

Borges, B. C. D., Catelan, A., Sasaki, R. T., Ambrosano, G. M. B., Reis, A. F., and
Aguiar, F. H. B. (2013). Effect of the Application of a Casein Phosphopeptide-
Amorphous Calcium Phosphate (CPP-ACP) Paste and Adhesive Systems on
Bond Durability of a Fissure Sealant. Odontology 101 (1), 52–59. doi:10.1007/
s10266-012-0062-5

Brackett, M. G., Li, N., Brackett, W. W., Sword, R. J., Qi, Y. P., Niu, L. N., et al.
(2015). Effect of Polymeric Microparticles Loaded with Catechin on the
Physicochemical Properties of an Adhesive System. J. Dentistry 40 (3), 173–180.
doi:10.1177/154405910708600115

Fialho, M. P. N., Hass, V., Nogueira, R. P., França, F. M. G., Tursi, C. P., Basting, R. T.
C., et al. (2019). Effect of Epigallocatechin-3-gallate: Gallocatechin on Bond
Durability at the Adhesive Interface in Caries-Affected Dentin. J. Mech.
Behav. Biomed. Mater. 91, 398–405. doi:10.1016/j.jmbbm.2018.11.022

Gotti, V. B., Feitosa, V. P., Sauro, S., Correr-Sobrinho, L., Leal, F. B., Stansbury,
J., W., et al. (2015). Effect of Antioxidants on the Dentin Interface Bond Stability
of Adhesives Exposed to Hydrolytic Degradation. J. Adhes. Dent. 17 (1), 35–44.
doi:10.3290/j.ad.133515

Hagerman, A. E., and Klücher, K. M. (1986). Tannin-protein Interactions. Prog.
Clin. Biol. Res. 213 (3), 67–76.

Hagerman, A. E., and Butler, L. G. (1980a). Condensed Tannin Purification and
Characterization of Tannin-Associated Proteins. J. Agric. Food Chem. 28 (5),
947–952. doi:10.1021/jf60231a011

Hagerman, A. E., and Butler, L. G. (1980b). Determination of Protein in Tannin-Protein
Precipitates. J. Agric. Food Chem. 28 (5), 944–947. doi:10.1021/jf60231a010

Hass, V., Luque-Martinez, I., Muñoz, M. A., Reyes, M. F., Abuna, G., Sinhoreti, M.
A., et al. (2016a). The Effect of Proanthocyanidin-containing 10% Phosphoric
Acid on Bonding Properties and MMP Inhibition. Dent Mater. 32 (3), 468–475.
doi:10.1016/j.dental.2015.12.007

Islam, M. S., Hirashii, N., Nassar, M., Yu, C., Otsuki, M., and Tagami, J. (2014).
Effect of Hesperidin Incorporation into a Self-Etching Primer on Durability of
Dentin Bond. Dental Mater. 30 (11), 1205–1212. doi:10.1016/j.dental.2014.08.371

Jang, E.-J., Cha, S.-M., Choi, S.-M., and Cha, J.-D. (2014). Combination Effects of Bacitracin with Antibiotics against Oral Pathogens. Arch. Oral Biol. 59 (11),
1233–1241. doi:10.1016/j.archoralbio.2014.07.008

Kang, K. A., Zhang, R., Piao, M. J., Chae, S., Kim, H. S., Park, J. H., et al.
(2012). Baicalein Inhibits Oxidative Stress-Induced Cellular Damage via Antioxidant
Effects. Toxicol. Ind. Health 28 (5), 412–421. doi:10.1080/10540436.2011.597088

Kaul, T. N., Middleton, E., Jr., and Ogra, P. L. (1985). Antiviral Effect of Flavonoids
on Long-Term Bonding Stability on Caries-Affected Dentin - ScienceDirect.
Dental Mater. 36 (9), 1151–1160. doi:10.1016/j.dental.2020.05.007

Du, X., Huang, X., Huang, C., Wang, Y., and Zhang, Y. (2012). Epigallocatechin-3-
gallate (EGCG) Enhances the Therapeutic Activity of a Dental Adhesive. J.
Dentistry 40 (6), 485–492. doi:10.1016/j.dental.2012.02.013

Duan, C., Matsumura, S., Kariya, N., Nishimura, M., and Shimono, T. (2007). In Vitro
antibacterial Activities of Scutellaria Baicalensis Georgi against Caricogenic
Bacterial. Pediatr. Dent. J. 17 (1), 58–64. doi:10.1093/pdj/2394(07)00996-4

Epasinghe, D. I., Yiu, C. K. Y., Burrow, M. F., Tay, F. R., and King, N. M. (2012).
Effect of Proanthocyanidin Incorporation into Dental Adhesive Resin on Resin-Dentine
Bond Strength. J. Dentistry 40 (3), 173–180. doi:10.1177/154405911108600113

Ferrazzano, G., Amato, I., Ingenito, A., Zarrilli, A., Pinto, G., and Pollio, A. (2011).
Plant Polyphenols and Their Anti-cariogenic Properties: a Review. Molecules 16 (2),
1486–1507. doi:10.3390/molecules16021486

La, V. D., Howell, A. B., and Grenier, D. (2009). Cranberry Proanthocyanidins
Stabilize the Adhesive Interface: a 2-Year In Vitro Study. J. Prosthet. Dent. 101 (6),
2009–2012. doi:10.1016/j.prosdent.2008.06.015

Mcalpine, J., et al. (2017). Biostability of the Proanthocyanidins-Dentin Complex
and Adhesion Studies. J. Dent Res. 96 (4), 412–460. doi:10.1177/
0022034516680586

Zhao et al. Plant Extracts Influence Dentin Bonding
## APPENDIX

| Search terms                                                                                                                                   |
|---------------------------------------------------------------------------------------------------------------------------------------------|
| **#1** Epigallocatechin gallate OR epigallocatechin-3-gallate OR epigallocatechin-3-O-gallate OR “EGCG cpd” OR epigallocatechin gallate          |
| **#2** Quercetin[MeSH]                                                                                                                       |
| **#3** Genipin                                                                                                                               |
| **#4** Proanthocyanidins[MeSH] OR “condensed tannin” OR “anthocyanidin polymer” OR procyanidin                                               |
| **#5** Naringenin                                                                                                                            |
| **#6** Hesperidin[MeSH] OR “hesperetin 7 rhamnoglucoside” OR “hesperetin 7 rutinoside”                                                      |
| **#7** “Crosslinking agent” OR “cross link”                                                                                                  |
| **#8** Plant extracts[MeSH]                                                                                                                  |
| **#9** #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8                                                                                          |
| **#10** Dental cements[MeSH] OR “dental adhesive” OR “luting agent” OR dentistry [MeSH]                                                      |
| **#11** Bond* AND strength                                                                                                                   |
| **#12** #9 AND #10 AND #11                                                                                                                   |