

INSTITUTO UNIVERSITÁRIO EGAS MONIZ

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

NATURAL ANTIBACTERIAL COMPOUNDS WITH POTENTIAL FOR INCORPORATION INTO DENTAL ADHESIVES – A SYSTEMATIC LITERATURE REVIEW

Trabalho submetido por
Ana Catarina Neves e Sousa
para a obtenção do grau de **Mestre** em Medicina Dentária

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RESUMO

Os adesivos dentários têm um papel fundamental na Dentisteria Operatória moderna, estando em constante evolução. No entanto, a formação de cáries secundárias, causadas pela infiltração bacteriana na interface adesivo-dentária, é um problema que ainda persiste. Apesar do seu potencial, a utilização de agentes antibacterianos sintéticos em adesivos levanta algumas preocupações relativas à sua segurança, levando a um aumento do interesse por alternativas naturais, que oferecem uma solução biocompatível, sustentável e eficaz. Deste modo, o objetivo desta revisão foi avaliar se a incorporação de compostos antibacterianos naturais em adesivos dentários poderia proporcionar uma atividade antimicrobiana eficaz, sem comprometer a sua integridade. Esta revisão sistemática foi conduzida de acordo com as diretrizes PRISMA 2020. Foram analisadas quatro bases de dados: PubMed, Scopus, EMBASE e Web of Science, sem restrições de idioma ou de data de publicação, até julho de 2024. Os critérios de inclusão abrangeram estudos *in vitro* que testaram a incorporação de compostos antibacterianos naturais em adesivos dentários, avaliando tanto a sua eficácia antibacteriana quanto as suas propriedades físico-químicas. Foi também realizada uma avaliação do risco de viés dos estudos incluídos. A maioria dos estudos analisados relataram uma atividade antibacteriana significativa, mantendo a integridade do adesivo, o que foi geralmente alcançado com concentrações mais baixas dos agentes naturais. No entanto, embora concentrações mais elevadas aumentem a eficácia antimicrobiana, estas tendem a comprometer as propriedades do adesivo. Esta revisão releva o papel promissor dos compostos antibacterianos naturais na melhoria da funcionalidade dos adesivos dentários, salientando igualmente a necessidade de investigação contínua para superar os desafios remanescentes.

Palavras-chave: Compostos naturais; Eficácia antibacteriana; Adesivo dentário; Biocompatibilidade

ABSTRACT

Dental adhesives are essential in modern restorative dentistry and are in constant evolution. However, challenges like secondary caries from bacterial infiltration at the adhesive-tooth interface persist. While synthetic antibacterial agents in adhesives show promise, concerns about their safety have increased interest in natural alternatives that offer a biocompatible, sustainable and effective alternative. Therefore, this study aimed to evaluate whether natural antibacterial compounds in dental adhesives can provide effective antimicrobial activity without compromising their integrity. This systematic review followed PRISMA 2020 statement guidelines. Four databases were screened: PubMed, Scopus, EMBASE and Web of Science, without language or publication date restrictions, until July 2024. Inclusion criteria were in vitro studies that added natural antimicrobial substances to dental adhesives and tested their antibacterial efficacy and physicochemical properties. A quality assessment of the selected studies was conducted. Most of the studies reviewed reported significant antibacterial activity while retaining the adhesive's integrity, which was generally achieved with lower concentrations of the natural agents. While higher concentrations increase antimicrobial effectiveness, they also tend to negatively impact the adhesive's properties. This review highlights the promising role of natural antibacterial compounds in enhancing the functionality of dental adhesives, while also pointing to the need for continued research to address challenges.

Keywords: Natural compounds; Antibacterial effect; Dental adhesive; Biocompatibility

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LIST OF ABBREVIATIONS

ADT – Agar diffusion test

CLSM - Confocal laser scanning microscopy

CMC – Carboxymethyl chitosan

CNSL - Cashew nut shell liquid

EGCG – Epigallocatechin-3-gallate

LDH - Lactate dehydrogenase enzymatic method

MMPs - Matrix metalloproteinases

MTT – 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide

PSA - Phenol–sulfuric acid method

qRT-PCR - Reverse transcription-quantitative polymerase chain reaction

RoB – Risk of bias

SEM - Scanning electron microscopy

XTT – 2,3-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide

I. INTRODUCTION

Dental adhesive technology has revolutionized many aspects of restorative dentistry, allowing for a more conservative approach as there is no need to remove unnecessary healthy tooth structure in order to provide mechanical retention (Breschi et al., 2018; Vaidyanathan & Vaidyanathan, 2009).

Over the last decades, dental adhesive systems have continually evolved with the aim of achieving effective bonding to enamel and dentin and long-lasting restorations with proper margin sealing (Perdigão, 2020). However, several obstacles remain to be overcome, as failure and the subsequent need for replacement of resin composite restorations is still a major concern (Demarco et al., 2017).

Secondary caries is described in the literature as the most common reason for replacing existing restorations, and usually occur due to the penetration and subsequent proliferation of cariogenic bacteria along microgaps between tooth tissue and the restoration (Eltahlah et al., 2018; Kopperud et al., 2012; Mjör et al., 2000).

The contraction stress resulting from the polymerization shrinkage of dental adhesives has been demonstrated to contribute to the formation of marginal microgaps at the adhesive-tooth interface. This phenomenon permits the progressive infiltration of salivary fluids and microorganisms into the restoration (Milosevic et al., 2011; Pinna et al., 2017).

Furthermore, the hydrolysis of the adhesive interface compromises the marginal integrity of composite restorations, thereby facilitating bacterial microleakage (Kermanshahi et al., 2010). The accumulation of biofilm on composite restorations represents a further issue, as these materials tend to accumulate more biofilm than other restorative materials (Beyth et al., 2007).

Moreover, the use of minimally invasive techniques in dentistry has increased. In some cases, the removal of carious tissue is not complete, particularly in deep lesions, in order to prevent damage to the pulp and preserve the tooth structure (Innes et al., 2016). However, this methodology may result in the retention of active bacteria within the dentin, which could ultimately lead to the failure of the restoration (Singhal et al., 2016).

In light of this problem, the research and development of antibacterial dental materials that inhibit the growth and accumulation of oral bacteria offer a promising

approach to reduce the occurrence of secondary caries (Chen et al., 2018; Wang et al., 2023). Dental adhesive systems are in direct contact with the tooth surface, which makes them an ideal material to possess antibacterial activity (Leyva del Rio et al. 2020).

Various studies have incorporated antimicrobial agents in dental adhesives, in which quaternary ammonium monomers (Cheng et al., 2013; Imazato et al., 1997; Xiao et al., 2009), silver nanoparticles (Aguiar et al., 2023; Wang et al., 2023), zinc methacrylate's (Eskandarizadeh et al., 2019; Henn et al., 2011) and chlorhexidine (Lima et al., 2022) are some of the most commonly described. The first successful antibacterial dentin primer resulted of the incorporation of MDPB, an antibacterial quaternary ammonium monomer that is currently present in the composition of a commercial dental adhesive, Clearfil™ SE Protect (Kuraray Noritake Dental Inc., Japan) (Imazato et al., 1997).

However, there has been a growing concern in regard of the biocompatibility and cytotoxic effects of some synthetic bactericidal agents used in dental materials (Shahi et al., 2019).

As a result, the interest in application of natural antibacterial products in dental materials has increased, as they constitute a promising alternative due to their biocompatibility and therapeutic properties (Budala et al., 2023). Additionally, natural compounds constitute a more sustainable and cost-effective option, presenting minimal side effects when administrated at appropriate doses (Seow et al., 2014). A natural product is defined by Umaru (2023) as a “substance synthesized by plants or animals, or chemical substances found in nature with particular pharmacological effects”.

1. Objective

The objective of this study was to conduct a systematic review of in vitro studies that evaluated the antibacterial efficacy of dental adhesive systems incorporating natural antibacterial compounds. The hypothesis to be tested was whether natural antibacterial compounds, when integrated into experimental or commercial dental adhesive systems, can provide effective antimicrobial activity without compromising the physicochemical properties of the adhesive.

II. MATERIALS AND METHODS

The present systematic review was conducted in accordance with the guidelines of PRISMA 2020 statement (Page et al., 2021). The protocol for this study was submitted to PROSPERO International Prospective Register of Systematic Reviews and registered under nr. CRD42024571079.

1. Research Question and PICO Method

The research question was defined using the PICO framework:

P (Population): In vitro studies focused on dental adhesive systems.

I (Intervention): Incorporation of natural antimicrobial substances into dental adhesive systems.

C (Comparison): Control groups of dental adhesive systems without the incorporation of natural antimicrobial substances.

O (Outcome): Antibacterial efficacy and physicochemical properties (specifically, degree of conversion and bond strength) of modified dental adhesives.

As a result, the following research question was formulated: What natural substances can be added to dental adhesives to improve their antibacterial properties without compromising their integrity and performance?

2. Search Strategy

The literature search was carried from 1 July 2024 to 18 July 2024. The electronic bibliographic databases selected included PubMed, Scopus, EMBASE and Web of Science. There were no language or publication date restrictions and an individualized search strategy was developed for each database (Table 1). All references resulting from the initial search were imported into the online platform *Rayyan — Intelligent Systematic Review* (Rayyan Systems Inc., Cambridge, MA, USA) (Ouzzani et al., 2016) to remove duplicates.

Table 1. Search strategy used in each database

Database	Search Strategy
PubMed	(antibacterial OR antimicrobial OR antibiotic OR anti-caries OR antiseptic OR anticaries) AND ("Dental Adhesive" OR bond OR primer OR adhesive) AND (dental OR "restorative dentistry" OR "dental restoration" OR "adhesive dentistry") NOT (review)
Scopus	ALL ((antibacterial OR antimicrobial OR antibiotic OR anti-caries OR antiseptic OR anticaries) AND ("Dental Adhesive" OR bond OR primer OR adhesive) AND (dental OR "restorative dentistry" OR "dental restoration" OR "adhesive dentistry") AND NOT (review))
EMBASE	(antibacterial OR antimicrobial OR antibiotic OR anti-caries OR antiseptic OR anticaries) AND ("Dental Adhesive" OR bond OR primer OR adhesive) AND (dental OR "restorative dentistry" OR "dental restoration" OR "adhesive dentistry") NOT review
Web of Science	ALL=((antibacterial OR antimicrobial OR antibiotic OR anti-caries OR antiseptic OR anticaries) AND ("Dental Adhesive" OR bond OR primer OR adhesive) AND (dental OR "restorative dentistry" OR "dental restoration" OR "adhesive dentistry") NOT (review))

3. Inclusion Criteria

The inclusion criteria were as follows: controlled in vitro studies that incorporated natural antimicrobial substances into dental adhesive systems and that tested the antibacterial efficacy and physicochemical properties of the modified adhesives, specifically degree of conversion and bond strength evaluations.

4. Screening and Data Extraction

Two reviewers, working independently, undertook a comprehensive analysis and selection process for all titles and abstracts of the articles retrieved from the search strategy. This was conducted in accordance with the pre-established inclusion criteria. Those articles that appeared to meet the eligibility criteria were selected for a full-text analysis. This process was conducted independently and in duplicate. In the event of any

discrepancies regarding the eligibility of the studies included, a consensus was reached through discussion, and, when necessary, a third reviewer from the team was consulted.

The data extracted from each selected study included the following: authors and publication date; natural antibacterial compounds used, as well as the concentrations applied and the materials they were incorporated into; methodology of the antibacterial activity assessment; specimens used; microorganisms tested; physicochemical properties of the modified adhesives tested; and main conclusions.

5. Risk of Bias Assessment

The quality assessment was conducted by two independent reviewers using the RoBDEMAT tool (Delgado et al., 2022), a recently developed risk of bias tool designed specifically for pre-clinical dental materials research. The tool assesses four domains: bias in planning and allocation, bias in sample/specimen preparation, bias in outcome assessment, and bias in data treatment and outcome reporting.

In the event of a discrepancy between the two reviewers' assessments, a third member of the team was consulted to facilitate a discussion and reach a consensus. The risk of bias (RoB) analysis of each study is presented in Table A1 (see Appendix A), with each signaling question classified as "reported," "insufficiently reported," "not reported," or "not applicable," in alignment with the RoBDEMAT tool guidance.

The data from Table A1 was adapted, with "reported" being classified as low risk, "insufficiently reported" as medium risk, and "not reported" as high risk. This adapted data was then uploaded to the *robvis* online tool (McGuinness & Higgins, 2021), which generated a Traffick Light Plot and a Summary Plot of the studies.

III. RESULTS

1. Study Selection

Figure 1 depicts the study selection process in a flowchart that was made following the PRISMA 2020 statement guidelines (Page et al., 2021). A total of 15 255 potentially relevant records were identified across the four databases. After removing duplicates, 11 396 studies remained. These articles were then screened by title and abstract according to the inclusion criteria. From this screening, 25 records were selected for full-text reading. This resulted in the exclusion of 12 studies, since 2 did not incorporate the natural compound into a dental adhesive system, 4 did not assess any physicochemical properties of the adhesive and 6 did not perform degree of conversion measurements. Therefore, a total of 13 studies were included in this systematic review.

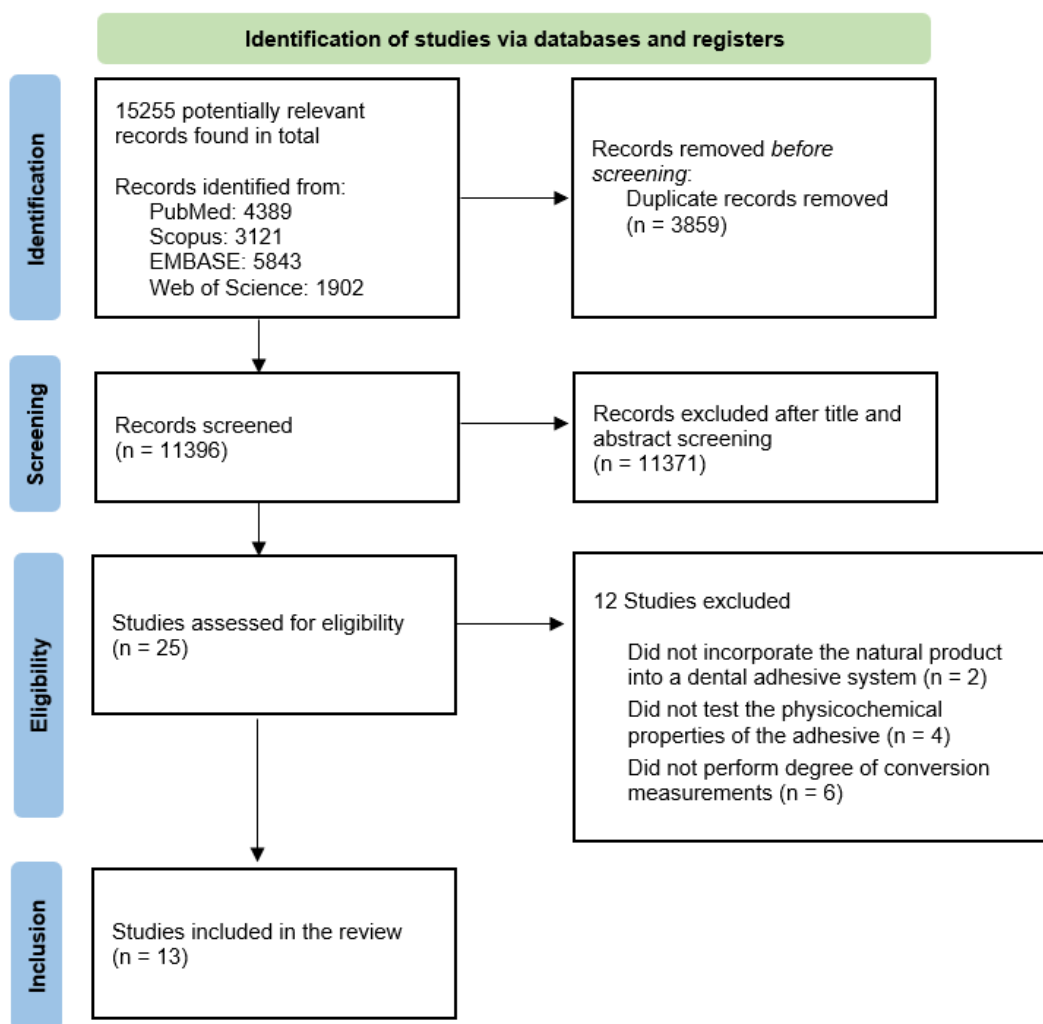


Figure 1. Flowchart outlining the study selection process (as described in the PRISMA 2020 statement guidelines)

2. Articles' General Features

Table 2 summarizes the general characteristics of the studies considered in this review in chronological order. Publication years of the articles vary from 2012 to 2022.

The studies examined a diverse range of natural antibacterial agents, 13 in total, with the majority being phenolic compounds. These include epigallocatechin-3-gallate (EGCG), found in green tea (Du et al., 2012); thymol, a key component of thyme essential oil (Rezaeian et al., 2019); and proanthocyanidin (Dias et al., 2020; Ribeiro et al., 2021), quercetin (Yang et al., 2017), and apigenin (Ribeiro et al., 2021), which are commonly present in various plant-based sources. Cashew nut shell liquid (CNSL) and its constituents, anacardic acid and cardol, were also part of this group (de Oliveira Souza et al., 2022). The other natural agents studied were nisin, an antimicrobial peptide produced by *Lactococcus lactis* (Su et al., 2018; Zhao et al., 2020); an essential oil derived from the *Butia capitata* palm tree (Peralta et al., 2013); chitosan, a natural polysaccharide biopolymer derived from chitin (Elsaka, 2012; Yao et al., 2022); arginine, an amino acid naturally produced by the human body (Geraldeli et al., 2017); and tt-farnesol, a natural sesquiterpene alcohol found in propolis (Leyva del Rio et al., 2020; Ribeiro et al., 2021).

The antibacterial agents were incorporated into both commercial and experimental dental adhesive systems. Common commercial adhesives used included Adper™ Single Bond 2 (Du et al., 2012; Elsaka, 2012; Su et al., 2018; Yang et al., 2017), Adper™ Scotchbond™ Universal (Leyva del Rio et al., 2020), Single Bond Universal (Zhao et al., 2020) and Ambar APS (de Oliveira Souza et al., 2022). Some studies utilized experimental formulations to adjust the adhesive properties by either adding the natural compound directly to the adhesive (Dias et al., 2020; Geraldeli et al., 2017; Peralta et al., 2013; Rezaeian et al., 2019) or incorporating it into an experimental primer (Ribeiro et al., 2021; Yao et al., 2022).

The concentration of the antibacterial agents exhibited considerable variation across studies, reflecting the differing antimicrobial strengths observed between compounds. The majority of the studies also tested different concentrations of the same agents in an effort to achieve an optimal balance between the highest antimicrobial efficacy and the maintenance of the adhesive's properties.

To evaluate the antibacterial activity of the modified adhesives a variety of methods were employed, with direct contact tests being the most frequently used among studies, followed by confocal laser scanning microscopy (CLSM) and XTT assays. Scanning electron microscopy evaluation (SEM), measurement of extracellular polysaccharides production and hardness loss of enamel and dentin are examples of some other tests employed to determine the adhesives' ability to inhibit bacterial growth and biofilm formation.

For these assays, specimens were chosen to simulate clinical conditions. Most studies conducted the antibacterial tests on adhesive discs or a combination of adhesive and composite discs. Only two studies used tooth tissue as substrates, specifically enamel discs coated with adhesive (Peralta et al., 2013) and enamel and dentin restorations (Ribeiro et al., 2021). The latter specimens provide a more realistic assessment of how the adhesive system would perform in a dental setting.

Streptococcus mutans was the primary microorganism tested in all studies, reflecting its role in the development of dental caries. Additionally, some studies evaluated the modified adhesives' antibacterial effects against other microorganisms, including lactobacilli and aciduric bacteria present in human saliva (Peralta et al., 2013), *Streptococcus gordonii* (Geraldini et al., 2017), *Candida albicans* (de Oliveira Souza et al., 2022), and the total microbial population in human saliva (Peralta et al., 2013; Zhao et al., 2020), to assess the broader antimicrobial spectrum of the agents.

The studies evaluated several key adhesive properties to ensure that the adhesive systems maintain their functionality after incorporating natural antibacterial agents. All studies performed degree of conversion and bond strength evaluations, as these were part of the inclusion criteria. Furthermore, some studies also assessed additional properties like flexural strength, modulus of elasticity, nanoleakage, viscosity, water sorption and solubility.

Table 2. Table summarizing the studies' characteristics

Author	Natural antibacterial agent	Incorporation material	Concentrations	Antibacterial effect assessment	Specimens	Microorganisms tested	Adhesive properties tested	Main conclusions
Du et al. (2012)	Epigallocatechin-3-gallate (EGCG)	Adper™ Single Bond 2 (SB) (3M ESPE, St. Paul, MN, USA)	100, 200, and 300 µg/ml	Direct contact test and SEM evaluation	Adhesive + composite discs	<i>Streptococcus mutans</i>	Microtensile bond strength and degree of conversion	Incorporation of EGCG at the concentration of 200 µg /ml exhibits antibacterial activity, whilst maintaining the adhesive integrity
Elsaka (2012)	Chitosan	Adper™ Single Bond 2 (3M ESPE, St. Paul, MN, USA)	0,12%, 0,25%, 0,5% and 1% (w/w)	Direct contact test	Adhesive discs	<i>Streptococcus mutans</i>	Microtensile bond strength, degree of conversion and viscosity	Experimental adhesive resin containing chitosan shows antibacterial effect. Incorporating 0,12% (w/w) chitosan does not adversely affect adhesive properties.
Peralta et al. (2013)	<i>Butia Capitata</i> essential oil	Experimental adhesive	1 mol%	Bacterial viability (colony counts) and pH supernatant	Enamel discs + primer and adhesive	Human saliva (<i>Streptococcus mutans</i> , lactobacilli, aciduric bacteria and total microorganisms)	Microtensile bond strength, degree of conversion, water sorption and solubility	<i>B. Capitata</i> oil presents similar antibacterial activity to a commercial antimicrobial adhesive, but also to the control. Adhesive properties were retained, however, there was a decrease in bond strength after 6 months.
Geraldeli et al. (2017)	Arginine	Experimental adhesive	7%	Direct contact test and CLSM	Adhesive discs	<i>Streptococcus mutans</i> and <i>Streptococcus gordonii</i>	Microtensile bond strength, degree of conversion, ultimate tensile strength, flexural strength, flexural modulus and Knoop hardness	Adhesive system containing 7% arginine exhibits antibacterial effects, while retaining physical and mechanical properties

Yang et al. (2017)	Quercetin	Adper™ Single Bond 2 (SB) (3M ESPE, St. Paul, MN, USA)	100, 500, and 1000 µg/ml	CLSM and XTT assay	Adhesive + composite discs	<i>Streptococcus mutans</i>	Microtensile bond strength, degree of conversion and nanoleakage	Adhesive modification with 500 µg/mL quercetin showed a balanced status of the antibacterial ability and adhesive properties
Su et al. (2018)	Nisin	Adper™ Single Bond 2 (3M, St. Paul, MN, USA)	1%, 3%, and 5% (w/v)	Film contact test, agar diffusion test, XTT assay and CLSM	Adhesive + composite discs Paper discs impregnated with the adhesive (ADT)	<i>Streptococcus mutans</i>	Microtensile bond strength and degree of conversion	A nisin-incorporated adhesive significantly inhibits the growth of <i>S. mutans</i> and its biofilm. However, concentrations above 1% exhibit a decrease in bond strength.
Rezaeian et al. (2019)	Thymol	Experimental adhesive	5 wt%	Direct contact test (based on ASTM E 2180–07)	Adhesive discs	<i>Streptococcus mutans</i>	Microshear bond strength, degree of conversion and flexural and viscoelastic properties	The thymol-incorporated adhesive showed appropriate antibacterial activity and comparable physico-mechanical properties to the control adhesive
Dias et al. (2020)	Proanthocyanidin	Experimental adhesive	1 wt%, 2 wt%, 4.5 wt%, and 6 wt%	Bacterial growth and MTT assay	Adhesive discs	<i>Streptococcus mutans</i>	Microtensile bond strength, degree of conversion, water sorption and solubility	The incorporation of proanthocyanidin did not promote an antibacterial effect in the adhesive.
Leyva del Rio et al. (2020)	Tt-farnesol	Adper™ Scotchbond Universal (3M ESPE, St. Paul, MN, United States)	0.38%, 1.90% and 3.80% (v/v)	Colony-forming units, biofilm dry weight, production of extracellular insoluble	Adhesive + composite discs	<i>Streptococcus mutans</i>	Microtensile bond strength, degree of conversion and hybrid layer permeability	Tt-farnesol increased antibacterial activity of the universal adhesive system. However, the degree of conversion and bonding

				polysaccharides and SEM				effectiveness of the adhesive were altered.
Zhao et al. (2020)	Nisin	Single Bond Universal (3M, St. Paul, MN, USA)	1%, 2% and 3% (w/v)	CLSM, qRT-PCR, PSA and LDH	Adhesive + composite discs	<i>Streptococcus mutans</i> and saliva-derived multispecies	Microtensile bond strength and degree of conversion	The incorporation of 3% (w/v) nisin in the adhesive achieved substantial antibacterial activity without compromising the bonding properties
Ribeiro et al. (2021)	Apigenin Proanthocyanidin Tt-farnesol	Experimental primer	4,5% proanthocyanidin, 1mM apigenin and 1mM apigenin + 5mM tt-farnesol	Hardness loss of enamel and dentin at the restorative margin	Enamel and dentin restorations	<i>Streptococcus mutans</i>	Microtensile bond strength, degree of conversion, nanoleakage, water sorption and solubility	Integration of apigenin and proanthocyanidin in a dental adhesive system showed promising results in preventing secondary caries in enamel and dentin, without compromising the adhesive physical properties. The association of apigenin + tt-farnesol decreased bond strength after 1 year and was not effective in reducing hardness loss in enamel
de Oliveira Souza et al. (2022)	Cashew nut shell liquid (CNSL) Anacardic acid Cardol	Ambar APS (FGM, Joinville, SC, Brazil)	15 µg/ml (each compound separately)	Direct contact test	Adhesive discs	<i>Streptococcus mutans</i> and <i>Candida albicans</i>	Microtensile bond strength, degree of conversion, elastic modulus, flexural resistance, water sorption and solubility	All 3 compounds showed antibacterial activity without jeopardizing the adhesive performance.

Yao et al. (2022)	Carboxymethyl chitosan (CMC)	Experimental primer	5, 10, and 20 mg/ml	Direct contact test, XTT assay and CLSM	Primer and adhesive + composite discs	<i>Streptococcus mutans</i>	Microtensile bond strength and degree of conversion	The incorporation of 20 mg/ml CMC obtained the highest antibacterial activity and did not adversely affect adhesive properties
SEM = Scanning electron microscopy; CLSM = Confocal laser scanning microscopy; qRT-PCR = Reverse transcription-quantitative polymerase chain reaction; PSA = Phenol-sulfuric acid method; LDH = Lactate dehydrogenase enzymatic method								

3. Studies' Patterns and Important Outcomes

3.1. Antibacterial Effectiveness

In general, the majority of the natural compounds studied showed significant antibacterial activity when incorporated into dental adhesives, which varied depending on the concentration and agent used.

Studies that tested different concentrations of these natural agents revealed a pattern of higher antimicrobial effectiveness at increased doses (Du et al., 2012; Leyva del Rio et al., 2020; Su et al., 2018; Yang et al., 2017; Yao et al., 2022; Zhao et al., 2020), with some compounds only showing significant antibacterial activity above certain concentrations. This was the case of EGCG (Du et al., 2012), nisin (Zhao et al., 2020) and tt-farnesol (Leyva del Rio et al., 2020), which only exhibited significant activity above 200 µg/ml; 2% (w/v) for *Streptococcus mutans* and 3% (w/v) for saliva-derived multispecies; and 1.90% (v/v), respectively. The only exception to this pattern was the study by Elsaka (2012), which found that chitosan provided statistically similar antibacterial effectiveness across all tested concentrations, showing no noticeable increase in antibacterial efficacy with higher doses.

Nonetheless, two studies did not report significant antibacterial effectiveness, as the results were not statistically different when compared to the control group. This was observed with the incorporation of proanthocyanidin by Dias et al. (2020) and *Butia capitata* oil (Peralta et al., 2013). Additionally, the combination of apigenin + tt-farnesol reduced hardness loss in dentin but not in enamel (Ribeiro et al., 2021).

3.2. Adhesive Integrity

The reviewed studies showed that natural antibacterial compounds can be successfully incorporated into dental adhesives without compromising their crucial properties. In cases where different concentrations of the natural agents were tested, lower concentrations tended to preserve the adhesive's properties, while higher doses often compromised adhesive integrity (Elsaka, 2012; Geraldeli et al., 2017; Su et al., 2018; Yang et al., 2017; Zhao et al., 2020). There were, however, some exceptions that deviated from this pattern, as EGCG was not only able to retain bond strength even at higher concentrations, but also increase microtensile bond strength at lower doses (Du et al., 2012); and both carboxymethyl chitosan (CMC) (Yao et al., 2022) and proanthocyanidin

(Dias et al., 2020) did not report differences in adhesive performance at higher concentrations.

On the contrary, the incorporation of tt-farnesol (Leyva del Rio et al., 2020) significantly reduced degree of conversion and bond strength, regardless of its concentration. Microtensile bond strength was also reduced with the association of apigenin + tt-farnesol after 1 year of aging (Ribeiro et al., 2021), and with *Butia capitata* oil after 6 months (Peralta et al., 2013). However, *Butia capitata* oil presented bond strength values after aging that were statistically similar to those of other commercial adhesives (Peralta et al., 2013).

3.3. Long-term Performance and Stability

While the studies showed promising short-term results, the long-term performance of these modified adhesives needs further investigation, as only 5 studies evaluated the antibacterial activity of the modified adhesives after aging and only 6 studies assessed their properties over time.

Regarding antimicrobial effectiveness, the aging period of the studies ranged from 5 days to 1 year. Despite some reduction over time, the adhesives generally retained their antibacterial activity at higher concentrations of the natural agents (Du et al., 2012; Elsaka, 2012; Rezaeian et al., 2019; Yang et al., 2017). Tt-farnesol, however, reported increased antimicrobial effectiveness after 5 days (Leyva del Rio et al., 2020).

Long-term adhesive stability was evaluated through microtensile bond strength and nanoleakage evaluations after aging. The results were varied: EGCG (Du et al., 2012) and CMC (Yao et al., 2022) maintained bond strength across all tested concentrations, while only 500 µg/ml quercetin (Yang et al., 2017) and proanthocyanidin above 1 wt% (Dias et al., 2020) showed similar outcomes. In contrast, *Butia capitata* oil (Peralta et al., 2013) and the combination of apigenin + tt-farnesol (Ribeiro et al., 2021) exhibited reduced bond strength after 6 months and 1 year, respectively. Nanoleakage evaluations revealed no increase after 1 month for any concentration of quercetin (Yang et al., 2017), but there was a significant increase with apigenin and the apigenin + tt-farnesol combination (Ribeiro et al., 2021).

4. RoB Analysis of the Studies

The results of the risk of bias analysis and the overall score for each selected study and for each domain are presented in Figures 2 and 3, respectively. All studies exhibited a considerable risk of bias, as none of them included a sample size calculation or blinding of the test operator. However, all studies provided an adequate control group and statistical analysis, with only one study (Leyva del Rio et al., 2020) exhibiting insufficient reporting of identical experimental conditions across groups. The degree of bias related to the standardization of samples, testing procedures, and outcome reporting exhibited variability among the studies, with a range from low to medium risk. The application of sample randomization was limited to the two studies (Peralta et al., 2013; Ribeiro et al., 2021) that utilized teeth as specimens for the evaluation of antibacterial activity.

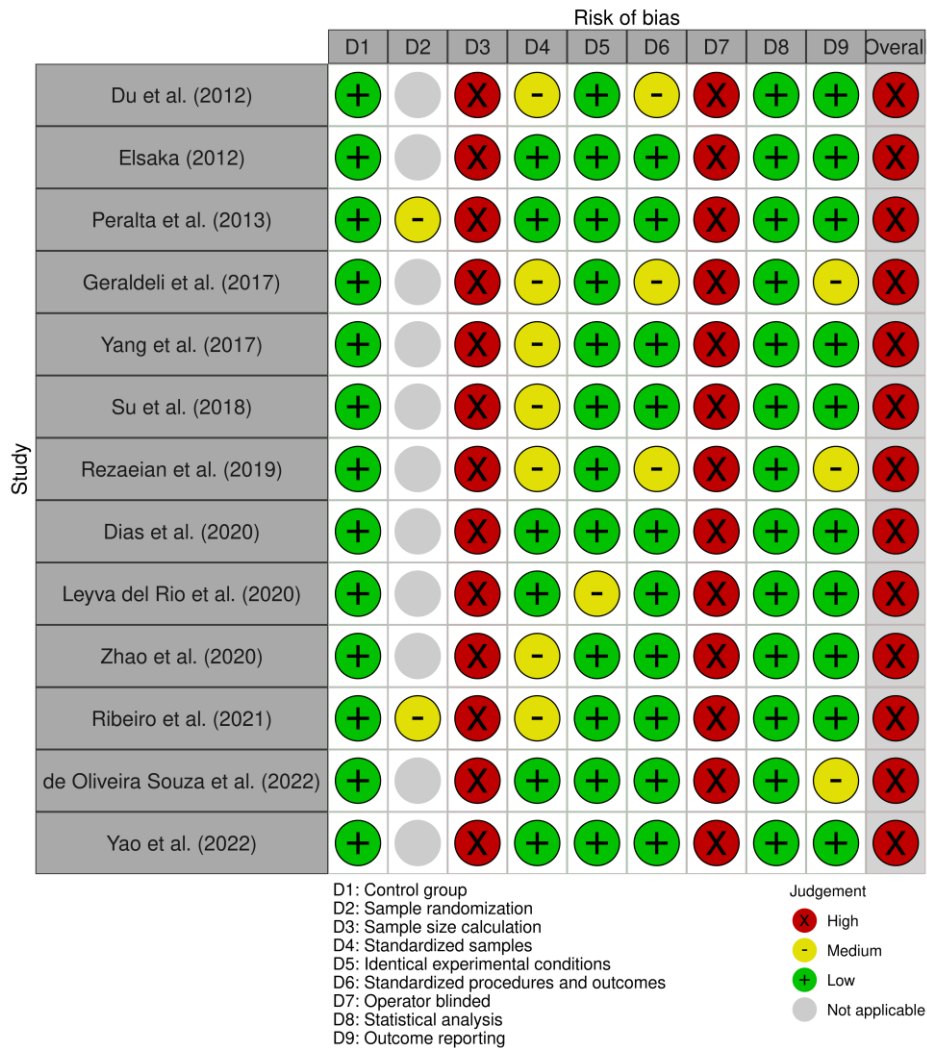


Figure 2. Traffick Light Plot for RoB analysis of each selected study in nine domains

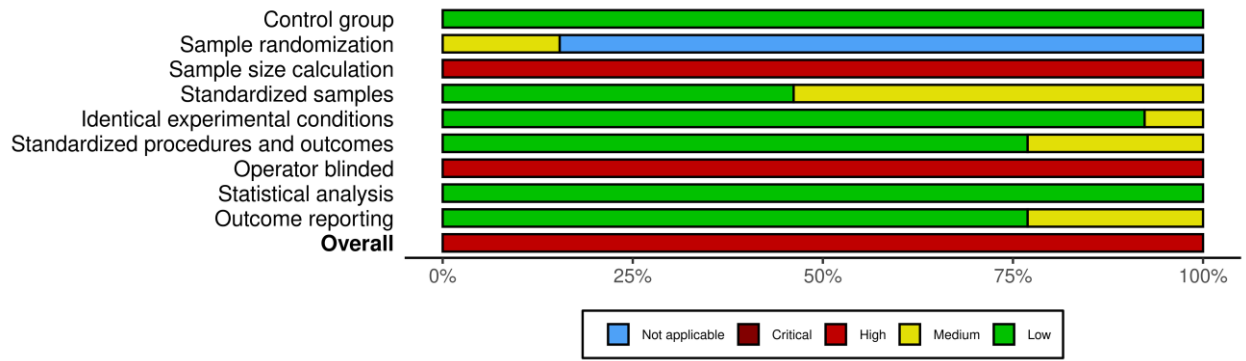


Figure 3. Summary Plot for RoB analysis of the studies

IV. DISCUSSION

1. Antibacterial Effectiveness

Dental caries is the most prevalent disease worldwide, estimated to affect over 2.5 billion people (Cherian et al., 2023). Resin composite restorations are a widely chosen treatment to replace decayed tissue, given that they offer excellent aesthetics and a less invasive cavity preparation (Demarco et al., 2012). Nonetheless, composite restorations often have a high failure and replacement rate, mostly attributed to bacterial microleakage at the tooth-restoration margins, leading to secondary caries (Spencer et al., 2010). A variety of synthetic antimicrobial agents have been studied and incorporated into experimental and commercial dental adhesives in order to try to overcome this problem, as it is the example of quaternary ammonium compounds, metallic nanoparticles and chlorhexidine (Chen et al., 2018).

However, despite being a less explored source of antibacterial agents for incorporation into dental materials, natural products could provide an alternative of interest to synthetic materials, due to their biocompatibility, sustainability and therapeutical properties (Wang et al., 2014).

Additionally, increasing concerns about health and the environment have driven a significant shift in consumer behavior, leading to a growing preference for more natural and biologic foods and materials, as people are becoming more conscious of the impact their choices have on their well-being and the planet (Paradowska-Stolarz et al., 2021).

This systematic review found consistent evidence across most of the studies selected that the incorporation of natural antibacterial compounds into dental adhesives can effectively inhibit bacterial growth and biofilm formation, especially from *Streptococcus mutans*, since it was the most tested microorganism. This suggests that these natural agents hold promise for enhancing the antimicrobial properties of dental adhesives, potentially reducing the occurrence of secondary caries and increasing the lifetime of composite restorations (Parashar et al., 2023).

The present review evaluated 13 different natural substances across 13 studies. Out of these studies, two failed to demonstrate a significant antibacterial activity of the dental adhesive modified by incorporating a natural agent.

Butia capitata oil demonstrated statistically similar antibacterial activity when compared to its control, despite also presenting similar values of bacterial growth inhibition to a commercial antibacterial adhesive containing MDBP, a quaternary ammonium monomer (Peralta et al., 2013). In this study, Peralta et al. (2013) only tested a singular concentration of the essential oil, which may lead to the hypothesis that a higher concentration could possess stronger antimicrobial effects, as this was a pattern observed among the other studies reviewed.

In contrast to those results, Peralta et al. (2017) conducted another study involving the incorporation of *Butia capitata* oil in a dental adhesive system, using the same concentration tested in 2013 (1 mol% of the essential oil) and the same experimental adhesive formulation. However, this time, the adhesive incorporated with the natural agent demonstrated a significantly higher antimicrobial performance than the experimental control adhesive and, once again, statistically similar values when compared to the same commercial antibacterial adhesive used in 2013 (Peralta et al., 2017). It is worth mentioning that this study from Peralta et al. (2017) was excluded from this review for not assessing the modified adhesive physicochemical properties, which was part of the inclusion criteria.

The incorporation of proanthocyanidin in an experimental dental adhesive by Dias et al. (2020) was also unable to enhance the antibacterial activity of the adhesive, since all concentrations tested (1 wt%, 2 wt%, 4.5 wt%, and 6 wt%) presented statistically similar levels of cell growth and metabolic activity inhibition of biofilm-forming bacteria between them and the control.

These results are contradicted by Ribeiro et al. (2021) that showed that the incorporation of 4,5% of proanthocyanidin in an experimental primer was able to reduce hardness loss in dentin and enamel when compared to the control, thus showing an increase of antibacterial effect. Nevertheless, the findings of Dias et al. (2020) also showed that, despite having similar values between them, all groups possessed a high antimicrobial effectiveness, even the control group, as a comparison was established with a commercial dental adhesive. Hence, it could be hypothesized that the antibacterial effect presented in the control was due to the low pH of the experimental adhesive, that ranged from 0,6 to 0,8, whereas the commercial adhesive also tested had a pH of 5,4 (Esteves et al., 2010). However, the antimicrobial effect achieved by the low pH of dental materials is considered limited in terms of bactericidal spectrum and durability (Imazato, 2003).

Additionally, the combination of apigenin + tt-farnesol was proven by Ribeiro et al. (2021) to present antibacterial effectiveness in the dentin, as it decreased hardness loss, but not in enamel. However, the same study demonstrated that incorporating only apigenin (in the same concentration) in an experimental primer reduced hardness loss both in dentin and enamel (Ribeiro et al., 2021). Another study included in this review showed that tt-farnesol could reduce *Streptococcus mutans* viability when incorporated into a dental adhesive at a concentration above 1,90% (v/v), but since the specimen used were composed of only adhesive + composite discs, it was not possible to understand the effects of tt-farnesol in enamel and dentin (Leyva del Rio et al., 2020).

Chitosan was evaluated in two different studies included in this review. Elsaka (2012) incorporated a chitosan solution (chitosan powder dissolved in acetic acid) into a commercial dental adhesive, while Yao et al. (2022) integrated carboxymethyl chitosan (CMC), a derivate of chitosan that is modified through carboxymethylation, into an experimental primer. Both studies reached the same outcomes, that modifying a dental adhesive system with chitosan can provide an effective antibacterial effect against *Streptococcus mutans* (Elsaka, 2012; Yao et al., 2022). Likewise, nisin was included in two of the studies reviewed, both of which reported an increase in antimicrobial activity due to the incorporation of the peptide into a dental adhesive (Su et al., 2018; Zhao et al., 2020).

Most of the studies evaluated the antibacterial activity of the modified dental adhesives solely against *Streptococcus mutans*, with only four studies including additional microorganisms (de Oliveira Souza et al., 2022; Geraldeli et al., 2017; Peralta et al., 2013; (Rezaeian et al., 2019). *Streptococcus mutans* is widely recognized in literature as a major cariogenic agent, significantly contributing to the development of dental caries and, therefore, its inhibition is crucial to the effectiveness of an antibacterial dental material (Loesche, 1986). Nonetheless, it is also important to evaluate this effectiveness against other microorganisms involved in the formation of dental caries, such as *Streptococcus sorbinus*, *Lactobacillus* spp. and *Actinomyces* spp, particularly in biofilms, since they exhibit greater resistance to antibiotics compared to planktonic cells (Faustova et al., 2018; Marcinkiewicz et al., 2013).

Despite the promising results in terms of antibacterial activity of these natural compounds, it is important to mention that only two studies used tooth substrates to perform these assays. Peralta et al. (2013) used bovine enamel and Ribeiro et al. (2021)

used human dentin and enamel. Without tooth substrates, it is impossible to accurately evaluate the effectiveness of antibacterial agents in preventing the formation of secondary caries (Sim et al., 2016). Therefore, further investigations are necessary to properly assess the potential antibacterial effectiveness of these natural compounds. Moreover, the use of different methodologies to test the antibacterial activity makes it challenging to compare the results across different studies, which highlights the need for a standardized method (Tobias, 1988).

2. Adhesive Integrity

One of the critical challenges in incorporating antibacterial agents into dental adhesives is preserving their integrity and stability, as any deterioration of the material's physicochemical properties could compromise the adhesive interface and lead to restoration failure (Farrugia & Camilleri, 2015).

Therefore, the assessment of these properties is crucial in the development of dental materials (Cocco et al., 2015), hence why it was included in the eligibility criteria of this review, specifically degree of conversion and bond strength evaluations.

A high degree of conversion has been correlated to a greater bond strength, biocompatibility and restoration durability, since an incomplete polymerization of the adhesive can result in unreacted monomers within the hybrid layer, creating a porous structure with reduced sealing ability and increased permeability, making it more susceptible to degradation (Cadenaro et al., 2005; Reus et al., 2010).

A bond strength test evaluates the adhesive's effectiveness in bonding to teeth, generally dentin, which is essential to ensure the clinical performance and longevity of dental restorations (Heintze et al., 2015). Additionally, other physicochemical properties like flexural strength, modulus of elasticity, nanoleakage, viscosity, water sorption and solubility were also assessed by some studies.

The studies reviewed showed that when natural antibacterial compounds were used at appropriate concentrations, they did not significantly impair essential adhesive properties. Only one study was a complete exception to this, as the incorporation of *tt*-farnesol (Leyva del Rio et al., 2020) significantly reduced degree of conversion and bond strength in all of the concentrations tested. As for nisin, the two studies included in this review reported slightly different outcomes in terms what concentrations were able to

preserve the adhesive's bond strength, since Su et al. (2018) reported that the incorporation of 3% (w/v) nisin decreased microtensile bond strength, while Zhao et al. (2020) found that the same concentration did not affect it.

Additionally, EGCG was found to increase bond strength at 100 µg/ml and even more at 200 µg/ml (Du et al., 2012). Several studies have reported the ability of EGCG to inhibit matrix metalloproteinases (MMPs) (Demeule et al., 2000; Yun et al., 2004). MMPs are proteolytic enzymes that break down exposed collagen fibers within dentin, resulting in the degradation of the hybrid layer and, consequently, decreased bond strength and durability (Pashley et al., 2004). Therefore, incorporating EGCG into dental adhesives could be a highly promising method for enhancing restoration longevity, as it combines antibacterial properties with reduced degradation of the adhesive interface.

3. Concentration Dependency

The effectiveness of both the antibacterial action and the maintenance of adhesive properties was closely linked to the concentration of the natural compounds used. This concentration dependency was evident across all studies that tested multiple concentrations.

Du et al. (2012) and Su et al. (2018) both found that the incorporation of natural substances only exhibited significant antibacterial effectiveness above certain concentrations, in alignment with the rest of studies reviewed, which indicated that higher quantities typically result in greater antimicrobial activity. However, these elevated concentrations were largely reported to negatively impact the adhesive's integrity and bonding performance, whereas lower doses tended to preserve these properties (Elsaka, 2012; Geraldeli et al., 2017; Su et al., 2018; Yang et al., 2017; Zhao et al., 2020).

Therefore, this indicates that there is a delicate balance between achieving sufficient antimicrobial efficacy and retaining the adhesive essential physicochemical properties. It also accentuates the importance of evaluating both the mechanical and biological characteristics of the dental adhesive, as well as multiple doses of the natural substances, in order to identify an optimal concentration that successfully meets both criteria (Garlet, 2013).

4. Long-term Performance and Stability

Although the short-term antibacterial effects and adhesive properties were generally favorable, the long-term performance of these modified adhesives remains a significant challenge. A dental adhesive with immediate antibacterial activity may be able to reduce residual bacteria present in the cavity; however, secondary caries often develops due to bacterial infiltration over time and the prolonged cumulative effects of their metabolism at compromised bond interfaces (Li et al., 2009). Aligned with that, it is also crucial that the adhesive is able to retain its mechanical properties over time, ensuring the stability and long-term clinical performance of restorations (Tezvergil-Mutluay et al., 2015).

In this review, the long-lasting antibacterial properties of the modified adhesives were tested in some studies after 7 days (Elsaka, 2012), 1 month (Du et al., 2012), 4 months (Rezaeian et al., 2019) and 10000 cycles (simulating 1 year of clinical physiological aging) (Yang et al., 2017). Overall, the antimicrobial activity decreased but remained effective compared to the control, particularly at higher concentrations, except for EGCG (Du et al., 2012), where effectiveness did not significantly decline after aging. Tt-farnesol's performance was also evaluated after 5 days, but its incorporation severely compromised the adhesive's properties, rendering it irrelevant (Leyva del Rio et al., 2020).

As for the long-term assessment of the physicochemical properties of the adhesives', 500 µg/ml of quercetin (Yang et al., 2017), EGCG (Du et al., 2012) and CMC (Yao et al., 2022) were successful in retaining bond strength after 1 month, 6 months and 10000 cycles of aging, respectively; as quercetin also retained nanoleakage levels after that period of time. In contrast, Peralta et al. (2013) documented a reduction in bond strength after 6 months for the adhesive containing *Butia Capitata* essential oil, whereas Ribeiro et al. (2021) noted a similar decrease for the adhesive containing apigenin + tt-farnesol after 1 year. Additionally, both apigenin and the combination of apigenin + tt-farnesol were reported to increase nanoleakage over time (Ribeiro et al., 2021).

These findings underscore the need for further investigation of the long-term performance of these natural compounds when incorporated into dental adhesives, since a prolonged antibacterial activity is crucial for reducing the occurrence of secondary caries. It is also relevant to further evaluate how these natural compounds interact with

the adhesive matrix over extended periods and under clinical conditions, as the decrease of bond strength over time may limit the practical application of these compounds, unless formulations can be adjusted to mitigate such effects.

5. Biocompatibility and Safety

Although not explicitly detailed in the summary, a key issue in incorporating natural compounds into dental adhesives is ensuring their biocompatibility and safety for patients. Natural does not always equate to safe, and introducing new compounds into dental materials requires thorough testing to confirm they do not cause adverse reactions or compromise patient health in other ways, especially for agents with bactericidal activity (Imazato et al., 1999).

Despite dentin acting as a barrier, its permeability increases when in close proximity to the dental pulp, making it crucial to assess potential toxic effects when developing novel dental materials, particularly regarding dental pulp cells, as they more accurately represent the *in vivo* target cells (Galler et al., 2005; Ohlsson et al., 2024).

Only two studies included in this review evaluated the cytotoxicity of the modified dental adhesives. Yang et al. (2017) conducted an MTT assay using human gingival fibroblast cells, finding no significant difference in cell viability after quercetin was incorporated at concentrations of 100 and 500 µg/ml. Similarly, Rezaeian et al. (2019) found that 5 wt% thymol maintained cell viability; however, this assay was performed using mouse fibroblast cells. These results indicate the low cytotoxicity and acceptable biocompatibility of these natural compounds for clinical use.

Although not assessed in the studies reviewed, most of the other compounds have undergone cytotoxicity testing in other investigations (Caiaffa et al., 2021; Frigaard et al., 2022; Peralta et al., 2017; Shariatinia, 2018; Shin et al., 2015).

This systematic review suggests that natural antibacterial agents could offer a more biocompatible and less cytotoxic alternative to synthetic chemicals (Antonucci et al., 2012; Chai et al., 2011; Liu et al., 2018). However, further research is necessary to fully confirm the cytocompatibility and safety of incorporating these compounds into dental adhesives.

6. Clinical Implications

The findings from these studies carry significant clinical relevance. Incorporating antibacterial properties into dental adhesive systems holds promise for reducing bacterial colonization around restorations, potentially lowering the incidence of secondary caries and extending the longevity of restorations (Chen et al., 2018; Kopperud et al., 2012).

However, successfully applying these findings in practice requires achieving a balance between antibacterial effectiveness and maintaining the adhesive's integrity and performance, ensuring long-term durability (Cocco et al., 2015). Additionally, ensuring a sustained antimicrobial effect and cytocompatibility is crucial (Imazato et al., 1999; Li et al., 2009). Meeting these criteria is essential for the successful development of formulations before they can be adopted in clinical settings (Garlet, 2013).

This review also contributes to the line of research focused on developing more natural, sustainable, and biological dental materials, aligned with a holistic approach to dentistry (Damle, 2016; Rathee & Vigarniya, 2018). Integrating these natural antibacterial substances into existing formulations could be a promising strategy for enhancing the therapeutic properties of adhesives.

Overall, the present systematic review underscored the promising role of natural compounds in providing antibacterial effectiveness when incorporated into dental adhesive systems. Hence, the tested hypothesis was accepted and the research question addressed, as the reviewed studies consistently demonstrated that incorporating natural antibacterial agents such as EGCG, chitosan, quercetin, thymol and nisin into either experimental or commercial dental adhesive systems effectively provided antimicrobial activity without adversely affecting the adhesive's physicochemical properties.

However, this evidence should be analyzed with caution due to the high risk of bias present in all the studies. Furthermore, many studies still need to assess the long-term performance and cytotoxicity of the modified adhesives, as well as their antibacterial activity against different microorganisms, and in tooth substrates. Therefore, despite the promising findings, it is clear that further research is required to address these limitations.

V. CONCLUSIONS

The growing concern for synthesizing more biocompatible and natural materials has been reflected in recent literature, highlighting a shift towards sustainable and health-conscious solutions in various fields, including dentistry. Hence, this review reports that natural antibacterial compounds have shown promising results in inhibiting bacterial growth and biofilm formation, especially against *Streptococcus mutans*.

Their integration into dental adhesives can provide a favorable strategy to reduce the occurrence of secondary caries, thereby reducing restorations failure and extending their longevity, without compromising adhesive integrity.

In the present systematic review, 200 µg/ml of EGCG, 500 µg/ml of quercetin and 20 mg/ml of CMC showed great potential in balancing effective antimicrobial activity while maintaining the adhesive's integrity and stability.

However, long-term performance, cytotoxicity and antibacterial activity of these modified adhesives against multiple microorganisms, remain areas requiring further investigation. Despite the encouraging results, ongoing research is essential to address these limitations and validate the in vivo efficacy and clinical use of these natural compounds.

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VII. APPENDICES

Appendix A

Table A1. RoB analysis of the studies using the RoBDEMAT tool

Author	D1: Bias in Planning and Allocation			D2: Bias in Sample/Specimen Preparation		D3: Bias in Outcome Assessment		D4: Bias in Data Treatment and Outcome Reporting	
	Control Group	Randomized Samples	Sample Size Calculation	Standardized Samples and Materials	Identical Experimental Conditions	Standardized Testing Procedures	Operator blinded	Statistical Analysis	Reporting Outcomes
Du et al. (2012)	Reported	Not applicable	Not reported	Insufficiently reported	Reported	Insufficiently reported	Not reported	Reported	Reported
Elsaka (2012)	Reported	Not applicable	Not reported	Reported	Reported	Reported	Not reported	Reported	Reported
Peralta et al. (2013)	Reported	Insufficiently reported	Not reported	Reported	Reported	Reported	Not reported	Reported	Reported
Geraldeli et al. (2017)	Reported	Not applicable	Not reported	Insufficiently reported	Reported	Insufficiently reported	Not reported	Reported	Insufficiently reported
Yang et al. (2017)	Reported	Not applicable	Not reported	Insufficiently reported	Reported	Reported	Not reported	Reported	Reported
Su et al. (2018)	Reported	Not applicable	Not reported	Insufficiently reported	Reported	Reported	Not reported	Reported	Reported

Rezaeian et al. (2019)	Reported	Not applicable	Not reported	Insufficiently reported	Reported	Insufficiently reported	Not reported	Reported	Insufficiently reported
Dias et al. (2020)	Reported	Not applicable	Not reported	Reported	Reported	Reported	Not reported	Reported	Reported
Leyva del Rio et al. (2020)	Reported	Not applicable	Not reported	Reported	Insufficiently reported	Reported	Not reported	Reported	Reported
Zhao et al. (2020)	Reported	Not applicable	Not reported	Insufficiently reported	Reported	Reported	Not reported	Reported	Reported
Ribeiro et al. (2021)	Reported	Insufficiently reported	Not reported	Insufficiently reported	Reported	Reported	Not reported	Reported	Reported
de Oliveira Souza et al. (2022)	Reported	Not applicable	Not reported	Reported	Reported	Reported	Not reported	Reported	Insufficiently reported
Yao et al. (2022)	Reported	Not applicable	Not reported	Reported	Reported	Reported	Not reported	Reported	Reported

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Received: 22 Sep 2024
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