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Exploring the effect of flavonoid-based solutions or formulations as antibiofilm agents in endodontics: A scoping review

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ABSTRACT

Objective: This scoping review aimed to map and summarize the evidence on the antimicrobial and antibiofilm efficacy of flavonoid-based solutions or formulations for endodontic applications.
Design: The review was conducted in accordance with the PRISMA-ScR guidelines. A comprehensive search was performed across databases for articles published up to April 2025. Eligible studies included those evaluating the antimicrobial effects of flavonoids on microorganisms associated with endodontic infections. Relevant data were extracted, and a descriptive synthesis of the findings was carried out.
Results: The results showed that fifteen studies met the inclusion criteria, demonstrating significant antimicrobial and antibiofilm activity of various flavonoids. Epigallocatechin-3-gallate was the most extensively studied compound, showing dose-dependent activity against *E. faecalis*, *F. nucleatum*, *A. israelii*, *S. mutans*, and *C. albicans*, with enhanced efficacy when combined with agents such as fosfomycin or the peptide KR-12-a5. Quercetin also displayed concentration-dependent antibiofilm effects and gene modulation in *E. faecalis*. Proanthocyanidin showed superior efficacy compared to conventional irrigants, especially at higher concentrations. Rutin, although limited in standalone efficacy, showed promising results when used as a photosensitizer. Other flavonoids such as apigenin, theaflavin, isoquercitrin, ampelopsin, chalcone, and chrysin also demonstrated varying degrees of antimicrobial activity, often similar to or surpassing conventional agents like chlorhexidine and calcium hydroxide in specific models.
Conclusions: Flavonoids exhibit significant potential as alternative or adjunctive agents for endodontic disinfection, especially in biofilm-related infections. Their efficacy is influenced by compound type, concentration, formulation, and synergistic combinations. Further research, especially clinical trials, is warranted to validate their therapeutic applicability and optimize delivery systems for clinical use in endodontics.

1. Introduction

Extensive dental caries or traumatic injuries can lead to pulp necrosis and infection of the root canal system (Smith, 2002). This system becomes a favorable environment for the establishment of a mixed, preponderantly anaerobic microbioma, which organizes into complex biofilms (Gomes & Herrera, 2018; Smith, 2002). The microorganisms and their metabolic products move along the system and reach the apical apex, activating a coordinated immuno-inflammatory interaction among cells within the periapical area (Márton & Kiss, 2000). The resulting host defense reaction can cause significant inflammation and damage to periapical tissues and can lead to apical periodontitis (Gomes & Herrera, 2018; Smith, 2002).

The treatment of apical periodontitis involves the chemical-mechanical instrumentation of the root canal, combined with use of intracanal medication for promoting disinfection of the root canal system (Arias et al., 2023; Dede et al., 2023; Sterzenbach et al., 2024; Weber et al., 2022). The endodontic management of immature

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permanent teeth, which have open apices, large root canals, thin dentinal walls, and short roots, becomes even more challenging, considering root canal therapy should disinfect root canal system and parallelly, promote complete root formation (Kahler et al., 2024).

Traditional treatment approaches, such as apexification with calcium hydroxide (CH) and apical plug/barrier with mineral trioxide aggregate (MTA), have been employed to manage infected immature teeth; however, these techniques do not result in further root maturation (Kahler et al., 2024). CH apexification includes the disinfection of the teeth with highly concentrated sodium hypochlorite (NaOCl) (2.5 – 6%) followed by placement of CH within the root canal. This procedure requires a long-term treatment (9–14 months) and periodical changes of CH. As a consequence of multiple appointments, tooth structure can become weak, increasing the risk of cervical root fractures and potentially compromising the long-term prognosis of the treated tooth (Asgary et al., 2024). The MTA apical plug technique aims to create an artificial barrier by placing MTA or other silicate-based cement at the root apex to facilitate root canal obturation (Asgary et al., 2024). Despite the advantages of the apical-plug therapy, such as shorter treatment time and better biocompatibility, neither of them enhances root formation, and tooth discoloration remains a notable drawback (Możyńska et al., 2017).

Recent research has focused on identifying biological materials with multiple therapeutic activities, including the ability of reducing microorganisms and inflammation in endodontically-compromised teeth, in addition to preserve Hertwig epithelial sheath (responsible for guiding the radicular dentin formation), the dental papilla and the odontoblast layer in the apical area, allowing the continuity in the root formation (Iglesias-Linares et al., 2013; Xie et al., 2021). Flavonoids are the most important classes of polyphenols, with more than 6000 compounds already described in the literature (Dias et al., 2021; Flemming et al., 2021; Schestakow et al., 2023). They are present in small amounts in fruits, seeds, and vegetables, generally as secondary metabolites and play important roles in plants specially against biotic and abiotic stresses (Dias et al., 2021). Studies have pointed out several pharmacological properties of polyphenols for humans, such as antimicrobial, antioxidant, anti-inflammatory, osteogenic, antiosteoclastogenic and other properties (Dias et al., 2021; Intharuksa et al., 2024; Yahfoufi et al., 2018).

Flavonoids have a common basic chemical structure (flavone), consisting of a 2-phenyl-1,4-benzopyrone nucleus with two aromatic rings (A and B) joined by three carbons that form a heterocyclic ring, called ring C. According to the variations in ring C, flavonoids are divided into flavones, flavonols, flavanones (3-hydroxy-flavone), flavanonol (2,3-dihydroflavonol), flavanols (or catechins), anthocyanidins and chalcones. Other types of compounds are included in the flavonoid group, the isoflavonoids and the neoflavonoids. Substitutions in the A and B rings give rise to different compounds within each class of flavonoids and flavonoids with the open C ring are called chalcones (Dias et al., 2021). Examples of flavonoids are: flavonols - quercetin, morin, myricetin, rutin, kaempferol, fisetin and isorhamnetin; flavanones – naringenin and hesperidin; flavones – luteolin, acacetin, vitexin and apigenin; isoflavone – genistein and diadzin; flavanols – catechin and epigallocatechin-3-gallate (EGCG), anthocyanidins – cyanidin and delphinidin (Nath et al., 2024).

The potential use of polyphenols for the prevention and treatment of oral diseases, especially dental caries and periodontitis, have been explored by many investigators (Beckman et al., 2024; Carneiro et al., 2024; Castellanos et al., 2024; Flemming et al., 2021; Hertel et al., 2017; Nunes et al., 2025). In Endodontics, the majority of the studies were focused on evaluating the antimicrobial and antibiofilm effects of different crude extracts containing flavonoids as potential agents for irrigation and intracanal medication (Borzini et al., 2016; Febvey et al., 2023). Few studies evaluated the antimicrobial activity of pure flavonoids against putative endodontic pathogens, particularly *Enterococcus faecalis (E. faecalis)* and *Candida albicans (C. albicans)* are in vitro or ex-vivo (in root dentin specimens) (Caiaffa et al., 2021; Duque et al., 2023; Graciani et al., 2023; Yang et al., 2020). In addition, flavonoids have shown low cytotoxicity and positive effects on dental stem cells, reducing reactive oxygen species production and inflammatory response stimulating, as well as, stimulating odontogenic differentiation and reparative dentin formation (Duque et al., 2022; Duque, Vizoto, et al., 2025; Elhakim et al., 2025; Liu et al., 2022; Mendes Soares et al., 2024).

Although flavonoids could be promising multifunctional agents for dental application, their low water solubility and degradability by oral fluids reduce their stability, bioavailability and bioefficacy when applied in solution. Therefore, natural or synthetic polymers have been used as drug carriers to promote the controlled release of compounds and extend their biological effect. Advantages of these carriers include reduction of doses or frequent exposure to antimicrobials, and the risk of bacterial resistance (Braga et al., 2022). Antimicrobial and antibiofilm effect of flavonoids-loaded drug delivery systems, such as hydrogels and nanoparticles, have been recently observed in in vitro and ex-vivo studies, showing an interesting strategy to reduce bacterial infection in root canals (Braga et al., 2022; Ferreira et al., 2025; Minhaco et al., 2023). However, the evidence remains fragmented. Therefore, a scoping review is warranted to systematically map the existing in vitro evidence, identify knowledge gaps, and provide a structured overview to guide future translational and clinical research. Thus, the objective of this study was to conduct a scoping review to evaluate the efficacy of flavonoid-based solutions or formulations as antimicrobial and antibiofilm agents in endodontic treatment.

2. Material and methods

2.1. Study protocol

This scoping review was carried out in accordance with the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) (Tricco et al., 2018), incorporating methodologies from recent publications (Nunes, de Oliveira Alves, Ragghianti,et al., 2025; Nunes, de Oliveira Alves, Peres, et al., 2025; Seron et al., 2024).

2.2. Eligibility criteria

To define the research question, the Patient, Concept, and Context (PCC) framework (Peters et al., 2015) was utilized, leading to the formulation of the following question: “Do flavonoids promote antimicrobial/antibiofilm effects when used in endodontic treatment?”

PCC Framework Details:

- Population: Studies evaluating the effects of flavonoids on microorganisms associated with endodontic infections. These include microbial samples/biofilms collected from the root canals of patients undergoing endodontic treatment, in vitro models using extracted teeth, infected root discs, dentin/dentin tubules, or direct exposure to planktonic microorganisms or biofilms.
- Concept: The application of flavonoids, in various forms—such as treatment solutions, incorporated formulations, irrigants, or intracanal medications. Comparison groups include negative controls (e.g., placebo or untreated samples) or positive controls using standard endodontic agents (e.g., NaOCl, chlorhexidine (CHX), Calcium hydroxide (CH), or other substances commonly employed in endodontic dental practice).
- Context: The primary outcomes of interest focus on antimicrobial efficacy, measured through: The total bacterial count. Positive bacterial culture results quantified as colony-forming unit (CFU/mL) or equivalent methods.

The inclusion criteria for this scoping review comprised in vitro, in silico, in situ, and in vivo (animal or human) studies assessing the antimicrobial or antibiofilm effects of flavonoids (excluding plant

extracts) in solution or injectable carrier systems for endodontic treatment. Eligible studies used pure flavonoids or those diluted in a vehicle, ensuring that the intervention differed only by the presence of flavonoids. There were no restrictions on publication period or language. Exclusion criteria included studies without a control group, those evaluating multiple antimicrobial agents that obscured the specific effects of flavonoids, and studies using plant extracts without isolating the tested flavonoid. Reviews and expert opinions were also excluded. In summary, any study failing to meet these predefined criteria was excluded from the review.

2.3. Search strategy

The electronic search was conducted across the following databases: PubMed (MEDLINE), Scopus, Web of Science, Embase, and Cochrane Library, up to April 10, 2025. Medical Subject Headings (MeSH) terms, developed with the assistance of a specialized librarian, were employed to construct the search strategy (see Appendix #S1) to enhance the sensitivity of the search. Free terms that were not available as descriptors in the databases were also included.

The search strategy was developed without restrictions on publication dates. To ensure comprehensive coverage, a manual search was performed to identify manuscripts potentially missed by the electronic search, focusing on articles published in key journals in the field, including: *Journal of Endodontics, International Endodontic Journal, Australian Endodontic Journal, Iranian Endodontic Journal, European Endodontic Journal, Restorative Dentistry and Endodontics, Journal of Dental Research, Clinical Oral Investigations, Journal of Dentistry, Dental Materials, International Dental Journal, Odontology,* and *Brazilian Oral Research*. Additionally, a manual screening of the reference lists of all included studies was conducted to capture any relevant articles that might have been overlooked.

2.4. Study selection

The article selection process began with a systematic evaluation of titles and abstracts, followed by a full-text evaluation of potentially eligible studies. In the first step, two independent reviewers (E.S.E. and G.P.N.) conducted an initial screening, importing all references retrieved from the databases into an online reference manager (EndNote Web; Thomson Reuters Inc., Philadelphia, PA, USA) to remove duplicates. If titles and abstracts provided insufficient information, the corresponding full texts were retrieved for a comprehensive review. Disagreements during this stage were resolved through discussion or consultation with a third reviewer (C.D.).

In the second step, after duplicates were removed, potentially relevant articles were screened for eligibility based on their titles and abstracts. Studies meeting the inclusion criteria underwent a detailed full-text review, with reasons for exclusion systematically documented. Final study selection was independently performed by the two reviewers, with a third reviewer resolving any conflicts to achieve consensus.

2.5. Data extraction and synthesis

Details of the studies were extracted using customized forms designed to capture comprehensive information, including the following parameters: authorship and year of publication, country of origin, study design, sample characteristics, details of study groups, evaluated microorganisms, assessment methods, results (outcomes), conclusions, and the effects of the interventions. Specific data regarding the types of flavonoids evaluated were systematically recorded (Table 1). When additional details were required, the corresponding authors were contacted via email for clarification.

The extracted data were synthesized into a narrative summary, highlighting experimental protocols, intervention characteristics, main findings, and conclusions, ensuring a concise and structured presentation of the results.

3. Results

3.1. Study selection, and characteristics of the included studies

A total of 478 studies were identified through database searches: PubMed (281), Scopus (79), Web of Science (64), Embase (35), and Cochrane Library (19). After the removal of 112 duplicates, 366 titles and abstracts were screened based on the predefined eligibility criteria. Nineteen articles were selected for full-text evaluation, and four were excluded for not meeting the inclusion criteria. As a result, 15 in vitro studies were included in this scoping review (Alipour et al., 2021; Aqabat et al., 2024; Braga et al., 2022; Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023; Graciani et al., 2023; Huang et al., 2023; Kim & Min, 2023; Lee & Tan, 2015; Liu et al., 2021; Pourhajibagher et al., 2024; Qayyum et al., 2019; Wang et al., 2024; Yang et al., 2020). (Fig. 1).

The analyzed fifteen articles were published between 2015 and 2025, and their characteristics are summarized in Table 1. The eligible studies originated from various countries, including Egypt (Aqabat et al., 2024), Iran (Alipour et al., 2021; Pourhajibagher et al., 2024), China (Huang et al., 2023; Kim & Min, 2023; Liu et al., 2021; Wang et al., 2024; Yang et al., 2020), Brazil (Braga et al., 2022; Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023; Graciani et al., 2023), India (Qayyum et al., 2019), and Singapore (Lee & Tan, 2015). All studies utilized an in vitro model to assess antimicrobial effects in endodontics.

3.2. Flavonoids and control agents

Several flavonoids were evaluated in the included studies, such as epigallocatechin-3- gallate (Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023; Lee & Tan, 2015), quercetin (Aqabat et al., 2024; Liu et al., 2021; Qayyum et al., 2019), proanthocyanidin (Huang et al., 2023; Yang et al., 2020), rutin (Braga et al., 2022; Pourhajibagher et al., 2024), apigenin (Kim & Min, 2023), theaflavin (Wang et al., 2024), chalcone (Graciani et al., 2023), chrysin (Alipour et al., 2021), ampelopsin (Braga et al., 2022), and isoquercitrin (Braga et al., 2022). The main control agents used in the included studies were CHX (Braga et al., 2022; Caiaffa et al., 2021; Duque et al., 2023; Graciani et al., 2023; Yang et al., 2020), sodium hypochlorite (Graciani et al., 2023; Huang et al., 2023; Pourhajibagher et al., 2024), calcium hydroxide (Aqabat et al., 2024; Chrisostomo et al., 2025), ethanol (Liu et al., 2021; Wang et al., 2024), and triple antibiotic paste (Chrisostomo et al., 2025). In addition, all studies included a negative control, consisting of untreated biofilms or those exposed only to sterile water or saline solution.

The concentrations of flavonoids tested varied across studies. Reported concentrations ranged from 12.5 to 200 μg/mL (Graciani et al., 2023; Lee & Tan, 2015; Pourhajibagher et al., 2024; Wang et al., 2024), 200–1000 μg/mL (Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023; Kim & Min, 2023; Lee & Tan, 2015), and above 1000 μg/mL (Braga et al., 2022; Kim & Min, 2023). Higher ranges were also tested, including 64–512 mg/mL (Qayyum et al., 2019), 1–2% (Aqabat et al., 2024; Liu et al., 2021), 3–6.5% (Alipour et al., 2021; Aqabat et al., 2024; Huang et al., 2023; Liu et al., 2021; Yang et al., 2020), and up to 10% (Yang et al., 2020). The concentrations of the main positive controls were as follows: CHX at 50–500 μg/mL (Braga et al., 2022; Caiaffa et al., 2021; Duque et al., 2023), and 2% (Graciani et al., 2023; Yang et al., 2020); calcium hydroxide at 41.667 μg/mL (Aqabat et al., 2024) and 1000 μg/mL (Braga et al., 2022; Chrisostomo et al., 2025); and sodium hypochlorite at 1% (Graciani et al., 2023) and 2.5–3% (Huang et al., 2023; Pourhajibagher et al., 2024).

The majority of the studies (73.3%, n = 11) evaluated the antimicrobial activity of flavonoids applied in aqueous or ethanol solution form (Caiaffa et al., 2021; Duque et al., 2023; Graciani et al., 2023;

Table 1
General data from the studies included in the scoping review.

Author/ year (Country)	Design study	Flavonoid (s)	Control (s)	Microorganisms tested	Form of Administration or use (Protocol)	Assessment Method	Main results	Intervention Effect
Chrisostomo et al. (2025) (Brazil)	*In vitro* Biofilms formed in specimens of bovine dentin roots	EGCG 0.625 mg/mL associated with fosfomycin (FOSFO) at 0.156 mg/mL in polyethylene glycol 400 (PEG)	Triantibiotic combination of metronidazole, ciprofloxacin and fosfomycin (TRI, all at 0.625 mg/mL), calcium hydroxide (CH, 1 mg/mL), PEG, and negative control (untreated cells)	*E. faecalis* (ATCC 51299), *S. mutans* (ATCC 25175), *A. israelii* (ATCC 12102), *F. nucleatum* (ATCC 25586)	Dentin root specimens were medicated with 500 µL of PEG alone or associated with EGCG+FOSFO, TRI or CH for 48 h and 7 days.	Analysis of stained live/dead cells by CLSM	After 48 h of treatment, the EGCG+FOSFO, TRI and CH groups had more than 80% dead cells in the multi-species biofilms, indicating an effective short-term antibiofilm effect, with no statistical differences between them. After 7 days of treatment, EGCG+FOSFO maintained the highest efficacy, with 82.9% of cells killed, followed by TRI (64.9%) and CH (54.5%). The study showed a superior long-term antibiofilm effect for EGCG+FOSFO.	Positive
Aqabat et al. (2024) (Egypt)	*In vitro* Antimicrobial assays in microplates Biofilms formed in specimens of human dentin roots	Quercetin 1.25%, 3% and 5% in Carbopol hydrogels	Calcium hydroxide (CH) and untreated biofilms	*E. faecalis* (ATCC 29212)	The root canals of the experimental groups were medicated with either an aqueous CH paste or quercetin hydrogels and sealed coronally with sticky wax for 7 days, while the root canals of the positive control group were sealed without being medicated.	MIC, MBC, CFU count, and live/dead staining analysis by CLSM	CH and quercetin used in the treatment model reduced microbial load in infected root canals fulfilled the ideal requirements of intracanal medications as being both potent antibacterial and, at the same time, non-cytotoxic.	Positive
Pourhajibagher et al. (2024) (Iran)	*In vitro* Antimicrobial assays in microplates Biofilms formed in specimens of human dentin roots	Solutions of Rutin-Ga (III) complex from 6.2 to 25 µmol/mL	Sodium hypochlorite (NaOCl), and untreated cells	*E. faecalis* (ATCC 29212)	*E. faecalis* biofilms were treated with a solution of rutin-Ga (III) complex at 6.2–25 µmol/mL for 5 min followed by the antimicrobial photodynamic therapy (aPDT) with energy density from 75 to 150 J/cm^2 for 75–150 s. The samples were incubated at 37°C for 24 h.	Minimum biofilm eradication concentration, minimum biofilm eradication dose, CFU count, SEM	Rutin-Ga (III) complex–mediated aPDT could effectively reduce the growth of *E. faecalis* biofilms formed inside root canals. Concentrations of this complex lower than 50 µmol/L had no significant effect in reducing the viability of microbial biofilms.	Positive
Wang et al. (2024) (China)	*In vitro* Antimicrobial assays in microplates Biofilms formed on 10 × 10 mm^2 glass coverslips	Theaflavin 7.81 µg/mL, 15.63 µg/mL and 31.25 µg/mL	Ethanol (0.016–4%) and sterile water	*E. faecalis* (ATCC 29212)	Theaflavin at different concentrations was added to 96-well plates containing the bacterial cultures and incubated for 24 h.	MIC, MBC, Crystal violet staining assay, CLSM, FE-SEM, transcriptomic analysis, qRT-PCR	Theaflavin significantly inhibited *E. faecalis* biofilm formation in a dose-dependent manner, altered transcriptomic profiles, downregulated quorum-sensing pathways, and suppressed key virulence genes (gelE, sprE, secY).	Positive
Duque et al. (2023) (Brazil)	*In vitro* Antimicrobial assays in microplates Biofilms formed in specimens of bovine dentin roots	EGCG at 0.625 mg/mL associated or not with FOSFO at 0.078 mg/mL	CHX 0.5 mg/mL Negative control (untreated biofilms)	*E. faecalis* (ATCC 51299), *S. mutans* (ATCC 25175), *A. israelii* (ATCC 12102), *F. nucleatum* (ATCC 25586)	Treatment of monospecies and multispecies biofilms formed in microplates, as well as multispecies biofilms formed in dentin tubules with EGCG, EGCG-FOSFO, CHX for 48 h	MIC, MBC, CFU/mL count, CLSM, SEM	EGCG and FOSFO showed strong antimicrobial activity, with a synergistic effect when combined, particularly against *A. israelii*, *F. nucleatum*, and *E. faecalis*. In mono- and multispecies biofilms, EGCG+FOSFO was as effective or superior to CHX in reducing bacterial load and disrupting the biofilm matrix. In the intratubular model, EGCG+FOSFO achieved the highest percentage of dead	Positive

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Table 1 (*continued*)

Author/ year (Country)	Design study	Flavonoid (s)	Control (s)	Microorganisms tested	Form of Administration or use (Protocol)	Assessment Method	Main results	Intervention Effect
							cells (84.85%), outperforming EGCG (78.49%) and CHX (50.6%).	
Graciani et al. (2023) (Brazil)	*In vitro* Biofilms formed on hydroxyapatite (HA) disks	Aminochalcone from 0.48 μg/mL to 156 μg/mL in an irrigant solution containing 0.2% menthol, 0.3% saccharin and 20% hydroalcoholic solution	0.2% CHX, 1% sodium hypochlorite and negative control (untreated biofilm)	*E. faecalis* (ATCC 29212) *C. albicans* (MYA 2876)	Endodontic irrigant containing aminochalcone tested on mono- and dual-species biofilms for 72 h	MIC, MBC/MFC, CFU/mL counts	The chalcone-based irrigant significantly reduced biofilm formation and viability, with MIC values of 7.8 μg/mL for *E. faecalis* and 15.6 μg/mL for *C. albicans*, without showing toxicity. Chalcone at 156 μg/mL significantly reduced mono and dual-species biofilms in HA disks, showing similar efficacy to 1% sodium hypochlorite, but lower than 2% CH	Positive
Huang et al. (2023) (China)	*In vitro* Biofilms formed in human dentin roots slices	Proanthocyanidin (PA) 6.5%	0.9% normal saline,17% EDTA + 3% NaOCl with or without carboxymethyl chitosan/amorphous calcium phosphate (CMC/ACP)	*E. faecalis* (ATCC 29212)	Endodontic irrigants: 6.5% PA associated or not with 17% EDTA + 3% NaOCl or CMC/ACP 5 mL od each irrigation solution was used for 10 min	SEM, CLSM	Proanthocyanidin combined with EDTA and NaOCl, as well with CMC/ACP showed significantly higher *E. faecalis* biofilm removal than the control group as examined by CLSM. In addition, PA associated with CMC/ACP preserved structural integrity of root dentin.	Positive
Kim and Min (2023) (China)	*In vitro* Antimicrobial assays in microplates Biofilms formed on hydroxyapatite (HA) disks (1.2 cm in diameter)	Apigenin 200–1000 μg/mL	Reduced Graphene Oxide (RGO) (180 μg/mL), Negative control (untreated biofilm)	*E. faecalis* (ATCC 47077)	Biofilms treated with apigenin with/without RGO for 1 h	MIC, MBC, CLSM, SEM, CFU/mL counts	Apigenin reduced *E. faecalis* biofilm viability in dose-dependent manner, showing bactericidal effects at higher concentrations, though with limited impact on biofilm biomass. When combined with RGO, its antimicrobial activity was significantly enhanced. Apigenin-RGO promoted biomass reduction and biofilm disruption, leading to superior effects compared to apigenin alone.	Positive
Braga et al. (2022) (Brazil)	*In vitro* Antimicrobial assays in microplates Biofilms formed in bovine dentin roots specimens	Ampelopsin (AMP) Isoquercitrin and Rutin from 1–0.0001 mg/mL and AMP at 2.5 mg/mL in poly (N-vinylcaprolactam) PNVCL hydrogel	Calcium hydroxide (CH) at 1 mg/mL, and chlorhexidine (CHX) at 0.5 mg/mL	*E. faecalis* (ATCC51299) *A. israelii* (ATCC12102), *Lactobacillus casei (IAL#523), S. mutans (ATCC 25175), F. nucleatum* (NCTC 11326)	Multispecies biofilms formed in microplates and inside root canals were treated with PNVCL hydrogels containing AMP, CH or CHX for 48 h	MIC, MBC, SEM, CLSM	Among the tested flavonoids, AMP showed the strongest antimicrobial activity on all oral bacteria tested. In multispecies biofilms, AMP loaded PNVCL hydrogel containing disrupted biofilm structure, reduced bacterial cells and extracellular matrix, with efficacy comparable to CHX and calcium hydroxide hydrogels. CLSM confirmed a higher proportion of dead intratubular cells in the PNVCL+AMP group (73.8%) versus CH (58.8%) and CHX (50.6%).	Positive

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Table 1 (*continued*)

Author/ year (Country)	Design study	Flavonoid (s)	Control (s)	Microorganisms tested	Form of Administration or use (Protocol)	Assessment Method	Main results	Intervention Effect
Alipour et al. (2021) (Iran)	*In vitro* Antimicrobial activity in agar plates Infected human dentin disks (4–5 mm of diameter and 1 mm thickness)	Chrysin incorporated into a poly-ε-caprolactone (PCL)-gelatin solution (5% w/w)	PCL/Gelatin: control scaffolds without chrysin	*E. faecalis (ATCC 13048), Cinetobacter baumannii (ATCC BAA-747), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 6538)*	Scaffolds of PCL/Gelatin/ Chrysin were placed onto agar media and to cover the infected dentin disks for 24 h.	Agar diffusion test and live/dead viabilty assay and CLSM analysis	The chrysin-loaded scaffolds reported antimicrobial and antibiofilm effects against the bacterial strains tested.	Positive
Caiaffa et al. (2021) (Brazil)	*In vitro* Antimicrobial assays in microplates Biofilms formed in microplates and in bovine dentin roots specimens	EGCG Serial dilutions from 0.00024 to 2 m/mL or at 0.6 mg/mL for biofilm assays or associated with the peptide KR-12-a5 at 0.3 mg/mL	Chlorhexidine (CHX) 0.05 and 0.5 mg/mL Negative control (untreated biofilm)	*E. faecalis* (ATCC 51299), *A. israelii* (ATCC 12102), *S. mutans* (UA159), *F. nucleatum* (NCTC 11326)	EGCG and other agents were tested in planktonic and biofilms assays in microplates, as well as in *E. faecalis* biofilms inside root canals.	MIC, MBC, CLSM, CFU/mL count	When combined with KR-12-a5, EGCG exhibited synergistic or additive effects against various bacteria, outperforming the individual agents. In biofilm models, the EGCG + KR-12-a5 combination achieved bacterial reduction comparable to CHX.	Positive
Liu et al. (2021) (China)	*In vitro* Biofilms formed in human dentin roots specimens	Quercetin at 1, 2, and 4% in ethanol	Sterile water and ethanol as control groups	*E. faecalis* (ATCC 29212)	A droplet (50 μL) of each quercetin solution and controls was placed on the pulp side of the dentin pieces for 3 min, and rinsed with water for 1 min.	CLSM	Both pure ethanol and quercetin solutions exhibited antibiofilm effects. The proportion of dead E. faecalis cells increased from 19.13% to 57.8% with increasing quercetin concentration.	Positive
Yang et al. (2020) (China)	*In vitro* Biofilms formed in human dentin roots specimens	Proanthocyanidin (PA) 2%, 5% and 10%	Sterile water and 2%CHX as controls	*E. faecalis* (ATCC 29212)	A droplet (50 μL) of each PA solution and controls was placed on the pulp side of the dentin pieces for 3 min and rinsed with water for 1 min.	CLSM	10% PA resulted in the highest percentage of dead cells. Lower concentrations of PA (2% and 5%) were comparable to CHX.	Positive
Qayyum et al. (2019) (India)	*In vitro* Antimicrobial and antibiofilm assays in microplates	Quercetin 32–512 mg/mL	Negative control (untreated biofilm)	*E. faecalis* (MTCC 2729)	Biofilms were treated with quercetin solutions for 24 h.	MIC, violet crystal, SEM, CLSM, MALDI TOF protein analysis, qRT-PCR	Quercetin inhibited *E. faecalis* biofilm formation in a dose-dependent manner at sub-MIC concentrations (64–256 mg/mL), reducing biofilm by up to 95% without affecting bacterial viability. CLSM and SEM confirmed decreased biofilm thickness without morphological damage. Proteomic analysis revealed 19 differentially expressed proteins linked to glycolysis, translation elongation, and protein folding, supporting quercetin's potential as a non-bactericidal antibiofilm agent and identifying molecular targets for future studies.	Positive
Lee and Tan (2015) (Singapore)	*In vitro* Biofilms formed in human dentin roots disks	Epigallocatechin-3-gallate (125–500 μg/mL) for biofilm assays	Control (untreated biofilm)	*E. faecalis* (ATCC 29212)	Biofilms were incubated with culture media containing EGCG for 7 days at 37°C	CFU/mL counts, CLSM, flow cytometry, qPCR	EGCG exhibited strong antimicrobial activity against *E. faecalis* (MIC = 5 μg/mL and MBC = 20 μg/mL) after 24 h. At 500 μg/mL, EGCG completely	Positive

(*continued on next page*)

Table 1 (*continued*)

Author/ year (Country)	Design study	Flavonoid (s)	Control (s)	Microorganisms tested	Form of Administration or use (Protocol)	Assessment Method	Main results	Intervention Effect
		5–20 μg/mL for other tests					eradicated mature biofilms on dentin discs, observed by bacterial counts and CLSM analysis. Additionally, even at subinhibitory concentrations (2.5 μg/mL), EGCG suppressed over 75% of the expression of key virulence genes (*ace*, *cylA*, *gelE*, and *sprE*), highlighting its potential as an anti-virulence agent.	

Abbreviations: *A. israelii: Actinomyces israelii;* CFU: Colony forming unit; *C. albicans: Candida albicans;* CLSM: Confocal laser scanning microscopy; CH: Calcium hydroxide; CHX: Chlorhexidine digluconate; *E. faecalis: Enterococcus faecalis;* EGCG: Epigallocatechin-3 gallate; FE-SEM: Field Emission-Scanning Electron Microscopy; FOSFO: Fosfomycin; *F. nucleatum: Fusobacterium nucleatum;* MBC: Minimum bactericidal concentration; MIC: Minimum inhibitory concentration; MFC: minimum fungicidal concentration; PA: Proanthocyanidin; qRT-PCR: Quantitative real-time polymerase chain reaction; SEM: Scanning electron microscopy; Sodium hypoclorite: NaOCl; *S. mutans: Streptococcus mutans.*

Huang et al., 2023; Kim & Min, 2023; Lee & Tan, 2015; Liu et al., 2021; Pourhajibagher et al., 2024; Qayyum et al., 2019; Wang et al., 2024; Yang et al., 2020). In contrast, only four studies incorporated flavonoids into polymers as delivery systems, such as hydrogels and scaffolds, targeting regenerative endodontic applications (Alipour et al., 2021; Aqabat et al., 2024; Braga et al., 2022; Chrisostomo et al., 2025). The exposure time of biofilms to flavonoids varied according to the proposed therapeutic purpose. When evaluated as alternative endodontic irrigants, shorter exposure times were used, ranging from 3 min (Liu et al., 2021; Yang et al., 2020), to 10 min (Huang et al., 2023), and up to 60 min (Kim & Min, 2023). In contrast, when flavonoids were investigated as intracanal medicaments, higher treatment durations were adopted, including 24 h (Alipour et al., 2021; Caiaffa et al., 2021; Pourhajibagher et al., 2024; Qayyum et al., 2019; Wang et al., 2024), 48 h (Braga et al., 2022; Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023), 72 h (Graciani et al., 2023), and up to 7 days (Aqabat et al., 2024; Chrisostomo et al., 2025; Lee & Tan, 2015).

3.3. Microorganisms and assessments

Regarding oral pathogens, all included studies evaluated the antimicrobial activity of flavonoids against *E. faecalis*. In addition, effects on other microorganisms were investigated, such as *Fusobacterium nucleatum* (Braga et al., 2022; Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023), *Actinomyces israelii* (Braga et al., 2022; Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023), *Streptococcus mutans* (Braga et al., 2022; Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023), *Candida albicans* (Graciani et al., 2023), *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Alipour et al., 2021), as well as *Lactobacillus casei* (Braga et al., 2022).

The experimental models varied in terms of biofilm complexity, including monospecies biofilms (Aqabat et al., 2024; Huang et al., 2023; Pourhajibagher et al., 2024; Wang et al., 2024; Kim et al., 2023; Alipour et al., 2021; Liu et al., 2021; Yang et al., 2020; Qayyum et al., 2019; Lee & Tan, 2015), multispecies biofilms (Braga et al., 2022; Chrisostomo et al., 2025), and both models (Caiaffa et al., 2021; Duque et al., 2023; Graciani et al., 2023). The biofilm development time also varied across studies: 24 h (Alipour et al., 2021; Braga et al., 2022; Lee & Tan, 2015; Pourhajibagher et al., 2024; Qayyum et al., 2019; Wang et al., 2024), 48 h (Braga et al., 2022; Caiaffa et al., 2021; Duque et al., 2023), 72 h (Duque et al., 2023; Graciani et al., 2023), and, in infected dentin root specimen models, 7 days (Aqabat et al., 2024; Chrisostomo et al., 2025; Huang et al., 2023; Lee & Tan, 2015; Liu et al., 2021; Pourhajibagher et al., 2024; Yang et al., 2020), 14 days (Braga et al., 2022; Caiaffa et al., 2021; Duque et al., 2023), and 21 days (Alipour et al., 2021).

The methods employed to evaluate the antimicrobial activity of flavonoids included both quantitative and qualitative approaches. The most commonly used techniques were: minimum inhibitory concentration (MIC) assays (Aqabat et al., 2024; Braga et al., 2022; Caiaffa et al., 2021; Duque et al., 2023; Graciani et al., 2023; Kim & Min, 2023; Pourhajibagher et al., 2024; Qayyum et al., 2019), minimum bactericidal concentration (MBC) assays (Aqabat et al., 2024; Braga et al., 2022; Caiaffa et al., 2021; Duque et al., 2023; Graciani et al., 2023; Kim & Min, 2023), live/dead staining combined with confocal laser scanning microscopy (CLSM) (Alipour et al., 2021; Aqabat et al., 2024; Braga et al., 2022; Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023; Huang et al., 2023; Kim & Min, 2023; Lee & Tan, 2015; Liu et al., 2021; Qayyum et al., 2019; Wang et al., 2024; Yang et al., 2020), colony-forming unit (CFU/mL) counts (Aqabat et al., 2024; Caiaffa et al., 2021; Duque et al., 2023; Graciani et al., 2023; Kim & Min, 2023; Lee & Tan, 2015; Pourhajibagher et al., 2024), agar diffusion tests (Alipour et al., 2021), quantitative real-time polymerase chain reaction (qRT-PCR) (Lee & Tan, 2015; Qayyum et al., 2019; Wang et al., 2024), and scanning electron microscopy (SEM) (Braga et al., 2022; Duque et al., 2023; Huang et al., 2023; Kim & Min, 2023; Pourhajibagher et al., 2024; Qayyum et al., 2019; Wang et al., 2024).

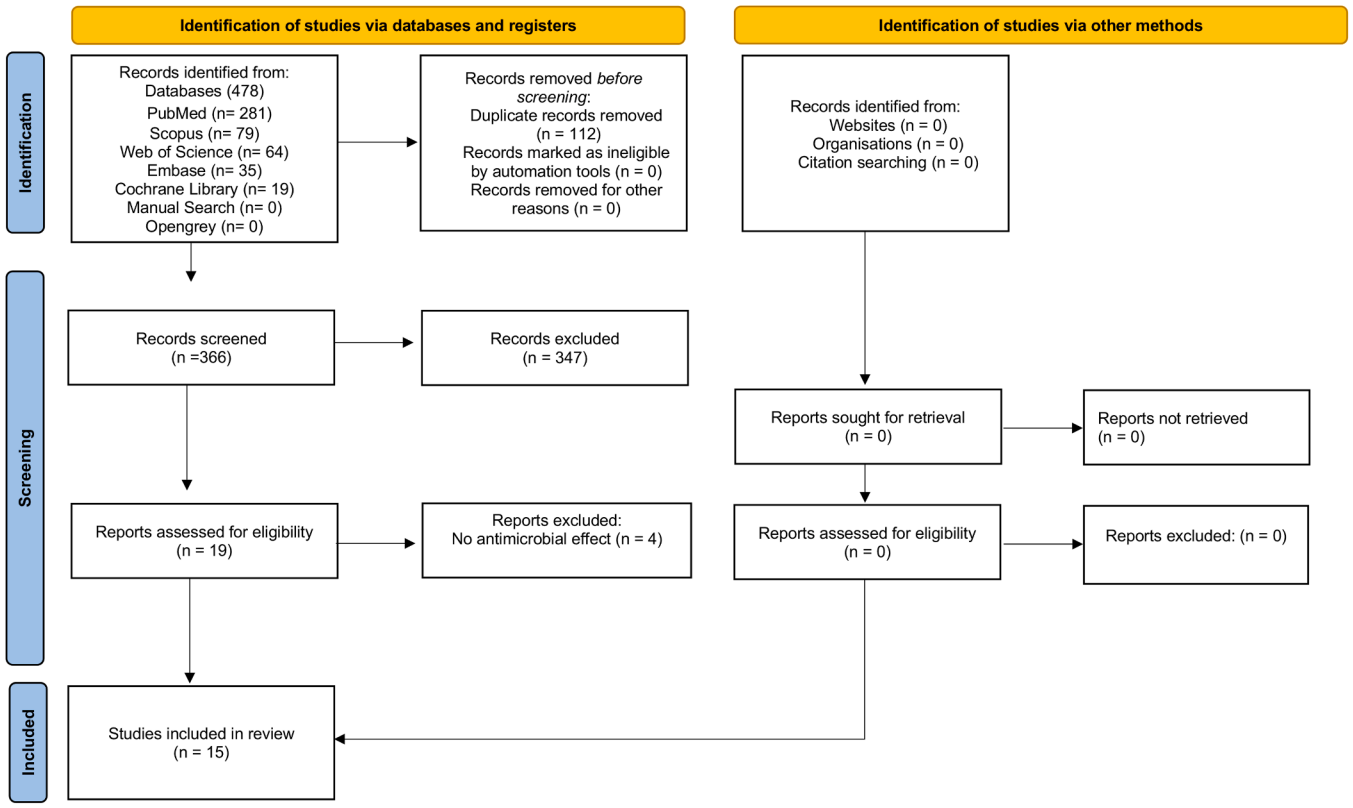

Fig. 1. Flow diagram presenting the identification of studies for the scoping review.

3.4. *Effects of flavonoids on oral pathogens associated with endodontic infections*

The 15 studies included in this scoping review demonstrated the antimicrobial activity of flavonoids against microorganisms commonly associated with endodontic infections. The specific characteristics of each flavonoid evaluated are detailed below, including their effects and comparisons with the respective positive and negative controls used in the assays.

3.4.1. *Epigallocatechin-3- gallate (EGCG)*

Epigallocatechin-3-gallate has stood out for its antimicrobial and antibiofilm properties against clinically relevant oral pathogens, including *E. faecalis*, *F. nucleatum*, *A. israelii*, *C. albicans*, and *S. mutans* (Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023). All studies evaluating EGCG reported significant antimicrobial activity compared to negative controls (Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023; Lee & Tan, 2015), with a dose-dependent effect demonstrated by Lee & Tan (2015). When compared to conventional antimicrobial agents, EGCG showed similar efficacy to the triple antibiotic paste and calcium hydroxide in reducing colony-forming units (CFU/mL) (Chrisostomo et al., 2025), and similar activity to CHX (Caiaffa et al., 2021; Duque et al., 2023). In dentin disk infection models with *E. faecalis*, EGCG outperformed CHX at concentrations 10 × and 100 × MIC (Duque et al., 2023), while another study reported similar efficacy to CHX at 0.05 mg/mL (Caiaffa et al., 2021).

Moreover, available studies indicate that the antimicrobial efficacy of EGCG can be significantly enhanced when combined with other agents, especially fosfomycin (FOSFO) and the synthetic peptide KR-12-a5, leading effects similar or superior to CHX in various experimental models (Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023). The EGCG+FOSFO combination exhibited potent antibiofilm activity against multispecies biofilms, with cell death rates exceeding 80% after 48 h of exposure and reaching 82.9% after 7 days – outperforming conventional formulations such as the triple antibiotic paste (64.9%) and calcium hydroxide (54.5%) (Chrisostomo et al., 2025). In an intratubular biofilm model, this combination achieved 84.85% dead cells, a performance similar to that of EGCG alone (78.49%) and significantly higher than CHX at 100 × MIC (50.6%) (Duque et al., 2023). These findings were supported by CLSM and SEM analyses, which revealed biofilm structural disruption and a marked reduction in the extracellular matrix.

Another investigated approach was the antimicrobial activity of EGCG, either alone or in combination with cationic peptides such as LL-37 and its analog KR-12-a5 (Caiaffa et al., 2021). EGCG showed significant antimicrobial effects, particularly against *A. israelii* and *F. nucleatum*. However, its effect against *E. faecalis* in intratubular biofilms was less pronounced when used alone. Despite this, a significant reduction in microbial viability was observed across all groups: 37.93% dead cells with EGCG alone and 88.04% with the EGCG + KR-12-a5 combination, compared to 49.72% and 89.41% achieved with CHX at concentrations of 0.05 and 0.5 mg/mL, respectively. The EGCG + KR-12-a5 combination demonstrated statistically superior efficacy compared to the isolated compounds and was equivalent to CHX at 0.5 mg/mL. Notably, the synergism promoted by KR-12-a5 was evidenced by low Fractional Inhibitory Concentration (FIC) values, significantly enhancing EGCG's antimicrobial action. This combination eliminated *S. mutans*, reduced *E. faecalis* by 5.52 log units, and achieved 88.04% dead cells in dentinal tubules – similar to 0.5 mg/mL CHX (89.41%) and superior to the individual agents.

3.4.2. *Quercetin*

Quercetin has demonstrated significant activity in inhibiting *E. faecalis* biofilms (Aqabat et al., 2024; Liu et al., 2021; Qayyum et al., 2019). The minimum inhibitory concentration of quercetin was 250 μg/mL – approximately six times higher than that of calcium hydroxide (41.667 μg/mL), while the minimum bactericidal concentration, at 125 μg/mL, was equivalent to that of calcium hydroxide,

suggesting that both compounds exhibit similar bactericidal potential at higher concentrations. Furthermore, quercetin outperformed calcium hydroxide in inhibiting biofilm formation, although it was less effective in reducing the total viable bacterial count (Aqabat et al., 2024).

In dentinal tubules infected with *E. faecalis*, quercetin exhibited a concentration-dependent antibiofilm effect, with cell death rates increasing from 19.13% to 57.8% as the concentration rose from 1% to 4%. The 4% concentration showed the highest antibiofilm efficacy, significantly outperforming both the negative control and ethanol (Liu et al., 2021). Additionally, quercetin demonstrated dose-dependent inhibition of biofilm formation, with MIC at 512 mg/L, achieving 70%, 85%, and 95% inhibition at concentrations of 64, 128, and 256 mg/mL, respectively. CLSM and SEM images at 64 mg/L confirmed disruption of the biofilm matrix and reduction in biofilm thickness, although no bactericidal activity was observed at subinhibitory concentrations. At the molecular level, quercetin also downregulated five genes associated with bacterial stress response, suggesting a potential modulatory effect on gene expression in *E. faecalis* (Qayyum et al., 2019).

3.4.3. Proanthocyanidin (PA)

The reviewed studies indicate that proanthocyanidin exhibits significant antibiofilm activity against *E. faecalis*, with efficacy directly related to the concentration used, demonstrating a clear dose-dependent effect (Huang et al., 2023; Yang et al., 2020).

In infected dentin disk models assessed by CLSM, 6.5% PA showed significantly greater antibiofilm activity against *E. faecalis* biofilms than 0.9% saline solution (control group) (Huang et al., 2023). When used alone, PA outperformed the conventional irrigation protocol combining 17% ethylenediaminetetraacetic acid (EDTA) and 3% NaOCl, indicating superior bacterial viability reduction. Furthermore, combining PA with EDTA and NaOCl enhanced the antibiofilm effect even more, surpassing the outcomes observed with either PA or the irrigants used in isolation. These findings were statistically confirmed by integrated optical density analysis, which revealed the highest antibiofilm activity in the combination group, followed by PA alone, and lastly by the conventional irrigants alone (Huang et al., 2023).

Additionally, in a similar experimental model, PA was compared with 2% CHX, considered the gold standard among endodontic irrigants. Results showed that 10% PA was the most effective solution in inactivating *E. faecalis* within dentinal tubules, significantly outperforming CHX. Moreover, 2% and 5% PA concentrations demonstrated antibacterial efficacy similar to CHX. CLSM analysis corroborated these findings, showing a higher proportion of dead bacterial cells in PA-treated groups, mainly at the 10% concentration (Yang et al., 2020).

3.4.4. Rutin

Rutin has demonstrated selective antibacterial activity against *F. nucleatum*, with a minimum inhibitory concentration of 0.5 mg/mL and a minimum bactericidal concentration of 1 mg/mL, while showing no efficacy against other tested pathogens such as *E. faecalis*, *A. israelii*, *L. casei*, and *S. mutans* (Braga et al., 2022). In contrast, when rutin was employed as a photosensitizer in photodynamic therapy, it significantly and dose-dependently reduced the viability of *E. faecalis* biofilms. Notably, concentrations of 12.5 μmol/L and 25 μmol/L resulted in reductions of 3.6 and 4.2 $\log_{10}$ CFU/mL, respectively ($p < 0.05$) (Pourhajibagher et al., 2024). Conversely, lower concentrations (6.2 μmol/L) did not significantly affect biofilm viability ($p > 0.05$). These outcomes were supported by field emission scanning electron microscopy (FESEM) analyses, which revealed marked biofilm disorganization and removal at higher rutin concentrations and longer irradiation times, whereas lower doses induced only mild morphological changes in bacterial cells (Pourhajibagher et al., 2024).

3.4.5. Other flavonoids: findings from isolated studies

Apigenin exhibited concentration-dependent bactericidal effects against *E. faecalis* biofilms, with minimum biofilm inhibitory concentrations (MBICs) ranging from 1to 2 mg/mL and minimum biofilm bactericidal concentrations (MBBCs) between 3 and 5 mg/mL. However, when used alone, its impact on biofilm biomass was limited. The combination of apigenin with reduced graphene oxide significantly enhanced its antimicrobial activity and biofilm-disrupting capacity, as confirmed by CLSM and MEV analyses, allowing comparable effects at reduced doses (Kim & Min, 2023).

Theaflavin also demonstrated promising activity, significantly inhibiting *E. faecalis* biofilm formation even at subinhibitory concentrations (sub-MICs). At 50% of the MIC (31.25 μg/mL), biofilm formation was suppressed by 97.53%. These findings were corroborated by CLSM and SEM analyses, which revealed reduced biofilm density and deformed bacterial cells. In addition, transcriptomic analyses identified differential expression of 248 genes, notably a downregulation of quorum sensing-related genes (*gelE*, *sprE*, and *secY*), suggesting that inhibition of bacterial virulence may be a potential mechanism of action of theaflavins (Wang et al., 2024).

Isoquercitrin and Ampelopsin exhibited antibacterial activity against *F. nucleatum*. Isoquercitrin showed MIC of 0.25 mg/mL and MBC of 1 mg/mL, while Ampelopsin presented MIC values ranging from 0.125 to 1 mg/mL and MBC values between 0.25 and 1 mg/mL, indicating substantial antimicrobial potential (Braga et al., 2022). Ampelopsin demonstrated antimicrobial efficacy similar to 1 mg/mL calcium hydroxide and 0.5 mg/mL CHX in multispecies biofilms. In SEM and CLSM analyses, hydrogels composed of poly(N-vinylcaprolactam) (PNVCL) combined with Ampelopsin induced significant biofilm disorganization, characterized by reductions in bacterial cells and extracellular matrix, similar to the effects observed for PNVCL + Calcium hydroxide and PNVCL + CHX. Quantitative analysis revealed a significant reduction in biofilm-associated bacteria in all treated hydrogel groups, with Ampelopsin resulting in the greatest reduction (73.8%), followed by Calcium hydroxide (58.8%) and CHX (50.6%) (Braga et al., 2022).

Another compound evaluated was chalcone, which demonstrated potent antimicrobial activity against *E. faecalis* and *C. albicans*. MIC and MBC/MFC values ranged from 7.8 to 15.6 μg/mL, indicating a microbicidal effect. In hydroxyapatite disk biofilm models, chalcone at 10 × MIC significantly reduced *E. faecalis* and *C. albicans* biofilms in both mono- and co-culture settings ($p < 0.05$), showing efficacy similar to 1% sodium hypoclorite, although 2% CHX exhibited superior effectiveness (Graciani et al., 2023).

Finally, chrysin incorporated into poly-ε-caprolactone (PCL) scaffolds at 5% demonstrated relevant antimicrobial activity against various strains – including *E. faecalis*, *A. baumannii*, *P. aeruginosa*, and *S. aureus* – with the highest efficacy observed against *E. faecalis*. Bacterial viability was reduced to below 16% ($p < 0.05$), in contrast to control PCL scaffolds without chrysin, which showed no significant antimicrobial effect (Alipour et al., 2021).

4. Discussion

Antimicrobial activity is a critical determinant of endodontic therapy success, especially in control of persistent microorganisms such as *E. faecalis*, which is frequently associated with treatment failure (Alghamdi & Shakir, 2020; Sharma et al., 2025). This scoping review, compiling and analyzed the available experimental evidence regarding the antimicrobial potential of flavonoids against microorganisms associated with endodontic infections. Overall, the included studies indicate that several flavonoids, such as EGCG, quercetin, proanthocyanidin and others have shown relevant antimicrobial and antibiofilm action in different in vitro models, in some cases comparable to or exceeding that of conventional agents such as sodium hypochlorite, CHX and Calcium hydroxide. However, it is essential to emphasize that these findings were obtained under highly heterogeneous and predominantly simplified experimental conditions. Most studies relied on in vitro models that do not fully reproduce the biological, structural, and ecological complexity of endodontic infections. Consequently, while the results consistently

demonstrate antimicrobial potential, their direct clinical applicability remains uncertain and should be interpreted with caution.

Medical research has increasingly focused on the role of biofilms in persistent bacterial infections, including endodontic infections. Flavonoids, plant-derived secondary metabolites produced in response to microbial stress, have shown broad antimicrobial activity in vitro, acting on multiple targets. They disrupt cell membranes, inhibit nucleic acid and protein synthesis, interfere with enzymes, and modulate virulence factors (Cushnie & Lamb, 2005; Wu et al., 2024). Specific flavonoids exhibit distinct actions: EGCG increases membrane permeability and induces oxidative stress (Capasso et al., 2025; Lee & Tan, 2015); quercetin compromises membranes and protein synthesis while exhibiting anti-inflammatory effects (Nguyen & Bhattacharya, 2022; Qayyum et al., 2019); proanthocyanidins destabilize membranes, inhibit adhesion, and block extracellular enzymes (Huang et al., 2023; Rauf et al., 2019; Yang et al., 2020). These mechanisms support antibiofilm efficacy against both mono- and multispecies biofilms, including early-stage and mature biofilms, and can be enhanced in combination with antibiotics, peptides, or advanced delivery systems. Flavonoids also show activity in the presence of infected dentin and at relatively low concentrations, suggesting a potential therapeutic window. All reported mechanisms and observed antimicrobial effects derive predominantly from simplified in vitro models, which cannot fully replicate the structural complexity, polymicrobial composition, metabolic interactions, and environmental conditions of clinical endodontic infections. Factors such as compound stability, penetration into complex root canal systems, interactions with dentin and host tissues, and the influence of the immune system may significantly alter efficacy in vivo. Consequently, while flavonoids present a biologically plausible and experimentally supported antimicrobial potential, their translation into clinically relevant outcomes remains uncertain and requires further investigation using robust in vivo and clinically representative models.

The findings of this scoping review reveal that flavonoids exhibit antimicrobial efficacy under various experimental conditions that mimic the complexity of endodontic infections. Included studies demonstrated that these natural compounds are effective against both monospecies biofilms – commonly formed by *E. faecalis* (Alipour et al., 2021; Aqabat et al., 2024; Huang et al., 2023; Kim & Min, 2023; Lee & Tan, 2015; Liu et al., 2021; Pourhajibagher et al., 2024; Qayyum et al., 2019; Wang et al., 2024; Yang et al., 2020) – and multispecies biofilms, which better represent the microbial diversity typically found in infected root canals (Braga et al., 2022; Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023; Graciani et al., 2023). Furthermore, flavonoids showed significant activity against both early-stage biofilms (Alipour et al., 2021; Braga et al., 2022; Lee & Tan, 2015; Pourhajibagher et al., 2024; Qayyum et al., 2019; Wang et al., 2024) and mature biofilms (Caiaffa et al., 2021; Duque et al., 2023; Graciani et al., 2023), which are known to be more resistant to antimicrobial penetration and frequently associated with treatment failure.

Notably, even in the presence of infected dentin roots – a biological substrate that can reduce the efficacy of conventional irrigants – flavonoids maintained their antimicrobial activity. This is particularly relevant considering the structural organization of biofilms and the inherent properties of dentin, such as its ability to absorb and neutralize active agents (Da Silva et al., 2013), potentially compromising clinical efficacy. Another noteworthy finding is the effectiveness of flavonoids at low concentrations (Braga et al., 2022; Caiaffa et al., 2021; Graciani et al., 2023; Lee & Tan, 2015; Pourhajibagher et al., 2024; Wang et al., 2024), with studies reporting bactericidal or viability-reducing effects at doses significantly lower than those required for conventional agents. Moreover, dose-dependent responses were observed (Braga et al., 2022; Huang et al., 2023; Liu et al., 2021; Yang et al., 2020), suggesting a favorable and adjustable therapeutic window. Collectively, these findings support the potential of flavonoids as versatile and effective agents, capable of acting at various stages of endodontic infection and under challenging clinical conditions, including mature biofilms and infected dentin substrates. Antimicrobial effects were observed against both early-stage and mature biofilms, the latter being particularly relevant due to their increased resistance to antimicrobial agents and frequent association with treatment failure. However, it should be noted that most of these findings derive from in vitro models that only partially reproduce the structural and biological complexity of clinical endodontic infections. Although some studies evaluated flavonoid activity in the presence of infected dentin, these models still represent simplified experimental conditions when compared with the in vivo environment. Several studies also reported antimicrobial effects at relatively low concentrations and demonstrated dose-dependent responses, suggesting a potential therapeutic window (Chrisostomo et al., 2025; Duque et al., 2023; Caiaffa et al., 2021). Nevertheless, such observations should be interpreted with caution, as concentration–effect relationships may differ substantially under clinical conditions. Collectively, these findings suggest that flavonoids hold experimental promise as antimicrobial agents; however, their effectiveness under clinically relevant conditions remains to be established through more robust models and in vivo investigations.

Moreover, studies included in this review, when evaluating the combined effect of flavonoids with other antimicrobials, such as antibiotics (e.g., fosfomycin) (Chrisostomo et al., 2025; Duque et al., 2023), synthetic peptides (e.g., KR-12-a5) (Caiaffa et al., 2021) and smart delivery systems (e.g., hydrogels or scaffolds) (Braga et al., 2022; Alipour et al., 2021), potentiate their antimicrobial effects through proven synergisms, as demonstrated by FIC indices, CLSM, and SEM analyses. Beyond their direct antimicrobial activity, flavonoids have also been shown to modulate bacterial gene expression, including stress response and quorum sensing genes, which may contribute to reducing microbial virulence and the formation of persistent biofilms (Liu et al., 2025). In advanced formulations such as nanoemulsions or controlled-release complexes, these natural compounds have demonstrated improved penetration into biofilms (Braga et al., 2022; Tran & Hadinoto, 2021), establishing themselves as multifunctional alternatives for the management of endodontic infections. Although not the primary focus of this review, 8 out of the 15 included studies investigated the biocompatibility of flavonoids through cytotoxicity assays on various cell lines, showing either no cytotoxic effects or only a slight reduction in cell viability when higher concentrations of the compounds were used (Alipour et al., 2021; Aqabat et al., 2024; Braga et al., 2022; Caiaffa et al., 2021; Chrisostomo et al., 2025; Duque et al., 2023; Graciani et al., 2023; Liu et al., 2021). These findings are highly relevant to contemporary endodontic therapy, which seeks effective, biocompatible, and sustainable alternatives to address treatment failures related to endodontic infection. Thus, the use of flavonoids represents an innovative strategy, offering advantages such as low cytotoxicity, multifactorial mechanisms of action, and a reduced likelihood of inducing bacterial resistance–highlighting their potential as adjuncts to conventional antimicrobials in the development of novel endodontic treatment protocols.

E. faecalis is frequently reported as one of the microorganisms associated with endodontic treatment failure, mainly due to its ability to survive under harsh environmental conditions and to persist within biofilm structures (Yang et al., 2024). However, it should be acknowledged that *E. faecalis* represents only one component of the complex and polymicrobial ecosystem typically found in persistent endodontic infections. In this review, it was noted that although widely used irrigants such as sodium hypochlorite and CHX exhibit recognized antimicrobial activity, the data analyzed indicate that these agents are not fully effective in eliminating mature *E. faecalis* biofilms (Clegg et al., 2006). Similarly, conventional intracanal medicaments, including CH, have shown limited efficacy against microorganisms organized in biofilms (Tronstad et al., 1981). Moreover, while sodium hypochlorite exhibits strong antimicrobial and tissue-dissolving properties, it is also highly cytotoxic and irritating to periapical tissues, with no residual antimicrobial effect or regenerative capabilities (Gomes et al., 2023; Yesilsoy

et al., 1995). CHX, although less toxic and known for its substantivity to dentin, lacks the ability to dissolve necrotic tissue and is less effective against deeper biofilm layers due to its interaction with the extracellular polymeric substances that protect the microbial community (Carrilho et al., 2010; Gomes et al., 2023; Ruiz-Linares et al., 2017; Yesilsoy et al., 1995).

In contrast, flavonoids represent a promising therapeutic alternative due to their multifaceted properties (Intharuksa et al., 2024; Liu et al., 2025; Salatin et al., 2022). Beyond their potent antimicrobial effects against resistant strains, flavonoids have demonstrated several biological activities not found in conventional irrigants and medicaments. Importantly, they stimulate alkaline phosphatase activity – a classical marker of osteoblastic differentiation – and promote mineralized nodule formation in human dental pulp cells and osteoblast-like cells (Braga et al., 2023; Duque, Rabelo, et al., 2025; Rabelo et al., 2025), indicating a potential role in tissue regeneration. These regenerative effects, combined with anti-inflammatory and antioxidant properties, may provide a favorable microenvironment for pulp and periapical tissue repair. Another key advantage of flavonoids is their superior biocompatibility, marked by low cytotoxicity and a reduced risk of inducing bacterial resistance, owing to their multitarget mechanisms of action. Furthermore, when formulated as nanoparticles or in controlled-release systems, flavonoids demonstrate enhanced biofilm penetration, improved stability, and sustained antimicrobial activity (Alipour et al., 2021; Braga et al., 2022; Kopka et al., 2023). Therefore, the incorporation of flavonoids into endodontic protocols represents a potentially promising investigative approach that addresses several limitations of conventional antimicrobial substances. Their reported antimicrobial, anti-inflammatory, and bioactive properties suggest possible advantages over traditional irrigants; however, these effects have been demonstrated predominantly under in vitro conditions. Although some studies indicate a potential influence on mineralization and cellular activity, required careful interpretation, as current experimental models do not adequately reproduce the biological complexity of the endodontic environment. Consequently, the translational relevance of these observations remains uncertain, and further studies using clinically relevant models are required before any conclusions can be drawn regarding their role in pulp tissue repair or regenerative endodontic applications.

This scoping review identified several limitations, particularly regarding methodological variability and the limited clinical relevance of the included studies. Differences were noted in flavonoid concentrations, exposure durations, delivery methods, antimicrobial assessment techniques, and experimental models. The antimicrobial activity was evaluated using diverse approaches, including assays on planktonic cultures, biofilm inhibition tests, CFU quantification, colorimetric metabolic assays (e.g., MTT, XTT), and structural analyses via CLSM or SEM, the diversity of methods complicates direct comparison of results and may limit the interpretation of antimicrobial efficacy. While CFU counts provide quantitative insights into microbial viability, they do not distinguish between bacteriostatic and bactericidal effects. Likewise, metabolic assays can be affected by residual compound activity, potentially misrepresenting microbial death. Although biofilm-based models are more representative of endodontic infections, only a minority of studies employed multispecies biofilms or clinically relevant settings. Many studies relied on simplified in vitro models, often focusing on monospecies biofilms, predominantly *E. faecalis*, which does not fully represent the polymicrobial complexity of clinical endodontic infections. Only a minority of studies employed multispecies biofilms or biologically relevant substrates such as infected dentin. Several clinically relevant aspects remain insufficiently investigated, including interactions with tooth structure and periapical tissues, effects on host immune response and oral cells, formulation stability, shelf-life, and handling properties. These limitations highlight that in vitro findings, while promising, may not directly translate to clinical efficacy and should be interpreted with caution.

Future studies should prioritize well-designed in vivo and clinical trials studies, employing multispecies biofilms, standardized protocols, and comprehensive cytotoxicity assessments. Additionally, investigating synergistic combinations of flavonoids with conventional irrigants or intracanal medicaments, as well as their incorporation into controlled-release or nanotechnology-based delivery systems, represents a promising strategy to enhance efficacy and safety as adjuncts in endodontic therapy.

5. Conclusion

Flavonoids, especially EGCG, proanthocyanidin, and quercetin, demonstrated strong and dose-dependent antibiofilm effects against endodontic pathogens, frequently approaching or even rivaling the activity of chlorhexidine under in vitro conditions. Nevertheless, important limitations currently hinder clinical translation. These include the lack of standardized and clinically relevant polymicrobial biofilm models, insufficient in vivo pharmacokinetic and toxicological data, and the absence of robust head-to-head comparisons with gold-standard irrigation and disinfection protocols. Future research should prioritize in vivo studies assessing the efficacy and safety of flavonoid-loaded delivery systems, particularly hydrogels such as PNVCL-based formulations, in infected animal tooth models. In addition, controlled investigations examining the adjunctive use of compounds such as proanthocyanidin in final irrigation protocols are warranted. Although promising, flavonoids remain a novel but still exploratory class of compounds. Well-designed translational and clinical studies are essential to determine their applicability in evidence-based endodontic practice.

CRediT authorship contribution statement

Renata Toledo Alves: Writing – review & editing, Visualization, Validation, Project administration, Investigation, Formal analysis, Conceptualization. **Marcelle Danelon:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis. **Torsten Sterzenbach:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Christian Hannig:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Cristiane Duque:** Writing – review & editing, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Gabriel Pereira Nunes:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elise Saint-Etienne:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anna Carolina Volpi Mello-Moura:** Writing – review & editing, Visualization, Methodology, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.archoralbio.2026.106559.

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