

Antibacterial Efficacy of Bromelain and Pineapple-Derived Extracts against *Aggregatibacter actinomycetemcomitans* and *Streptococcus sanguinis*: A Systematic Review

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ABSTRACT

Periodontal disease is a highly prevalent, biofilm-driven inflammatory condition, with severe periodontitis ranked among the most prevalent health conditions worldwide. Within the oral biofilm, *Streptococcus sanguinis* acts as an early coloniser that facilitates bacterial adhesion, while *Aggregatibacter actinomycetemcomitans* is implicated in the initiation and progression of periodontitis. Concerns regarding the adverse effects of long-term chlorhexidine use and rising antimicrobial resistance have stimulated interest in naturally derived alternatives. Bromelain, a proteolytic enzyme derived from *Ananas comosus* (pineapple), has demonstrated antibacterial, antibiofilm, and anti-inflammatory properties. This systematic review aimed to evaluate and synthesise evidence on the antibacterial efficacy of bromelain and pineapple-derived extracts against *A. actinomycetemcomitans* and *S. sanguinis*. The review was prospectively registered in PROSPERO (CRD420261497522) and conducted according to the PRISMA 2020 Statement. PubMed/MEDLINE, Scopus, ProQuest, and Google Scholar were searched from inception to 19 July 2026 without restrictions on publication year or language. Of 789 records identified, five *in vitro* experimental studies published between 2014 and 2025 met the eligibility criteria, while no eligible *in vivo* animal study was identified. Four studies evaluated preparations against *A. actinomycetemcomitans*, showing variable antibacterial activity, whereas one study demonstrated stronger activity of pineapple peel extract against *S. sanguinis*. No study achieved low risk of bias; four were rated medium risk and one high risk. Overall, bromelain and pineapple-derived extracts showed promising but inconsistent antibacterial activity, influenced by the plant part and extraction method. Further standardised studies and well-designed clinical trials are required before clinical recommendations can be made.

Keywords: *Aggregatibacter actinomycetemcomitans*; *Ananas comosus*; *Streptococcus sanguinis*; Bromelain; Medicine

INTRODUCTION

Periodontal diseases are among the most prevalent chronic inflammatory conditions affecting the oral cavity worldwide. Global burden-of-disease estimates indicate that severe periodontitis affected approximately 743 million people in 2010, making it the sixth most prevalent health condition globally, with an age-standardised prevalence that remained essentially unchanged between 1990 and 2010 [1]. Gingivitis and periodontitis are initiated and sustained by the accumulation of pathogenic dental biofilms on the tooth surface and within the gingival sulcus. Within this biofilm community, *Streptococcus sanguinis* functions as an early coloniser that facilitates the adhesion and subsequent colonisation of later, more pathogenic species, and has itself been implicated in biofilm gene expression relevant to gingivitis [2].

Aggregatibacter actinomycetemcomitans, by contrast, is recognised as one of the principal periodontal pathogens associated with the initiation and progression of periodontitis, expressing a range of virulence factors under different environmental conditions that promote tissue destruction [3]. Both species express virulence factors that contribute to bacterial adhesion and biofilm maturation, making them relevant targets for the development of novel antibacterial agents. Consistent with this concept, fractionated ethanolic extract of red ginger has been shown to exhibit promising antibacterial activity against *A. actinomycetemcomitans* by targeting virulence-associated proteins involved in bacterial adhesion and biofilm formation, both *in silico* and *in vitro*, highlighting the potential of natural products as alternative antibacterial agents for periodontal pathogens [4].

Conventional periodontal therapy primarily relies on mechanical plaque control supplemented with chemical antimicrobial agents such as chlorhexidine. Although chlorhexidine remains the gold-standard adjunctive antimicrobial in periodontal therapy, a recent systematic review comparing natural and conventional oral hygiene products noted that prolonged use is associated with undesirable adverse effects, including tooth staining, taste alteration, and mucosal irritation, and found comparable efficacy for several natural alternatives [5]. Current guidance advises chlorhexidine mouthwash for short-term use of two to four weeks only, which limits its suitability for patients who need longer-term chemical plaque control, such as those who find mechanical tooth brushing physically difficult, and in chemotherapy patients with neutropenia and mucositis, its use has even been associated with greater mucosal inflammation [6]. Antimicrobial resistance has also emerged as a concern, as oral microorganisms may adapt to chlorhexidine and cross-resistance to antibiotics has been reported [6]. These limitations have encouraged the exploration of naturally derived therapeutic agents as safer alternatives or adjunctive treatments. Plant-derived formulations delivered through mucoadhesive systems have, for example, demonstrated favourable immunomodulatory effects in experimental periodontitis models: a mucoadhesive gingival patch loaded with epigallocatechin-3-gallate (EGCG) modulated interleukin-6 and interleukin-10 expression in an *in vivo* periodontitis model [7], while a nano-emulsion of mangosteen (*Garcinia mangostana*) rind extract incorporated into a mucoadhesive patch produced regenerative periodontal effects in a further *in vivo* study [8]. These findings collectively show that *in vivo* evidence is both attainable and informative for natural periodontal therapeutics, motivating the present review's *a priori* intention to search for *in vivo* and *in vitro* evidence.

Bromelain, a mixture of proteolytic enzymes extracted from *Ananas comosus*, has attracted increasing attention because of its antibacterial, antibiofilm, anti-inflammatory, and wound-healing properties. A recent review of natural antimicrobials noted that bromelain, alongside the bacteriocin nisin, can inhibit bacterial growth, reduce bacterial adhesion, interfere with biofilm formation, and disrupt bacterial surface structures and extracellular polymeric substances, giving it broad-spectrum potential against Gram-positive and Gram-negative organisms alike [9]. These properties suggest that bromelain may serve as a promising natural antimicrobial agent, particularly against *S. sanguinis*, an early coloniser, and *A. actinomycetemcomitans*, a key periodontal pathogen.

Further evidence supports the potential application of natural products as antibiofilm and antiplaque agents in oral healthcare. A mouthwash formulated with *Zanthoxylum acanthopodium* (andaliman) extract significantly reduced the colony counts of *S. sanguinis* and *Staphylococcus aureus*, two important bacterial species involved in dental plaque formation [10]. Likewise, a herbal denture cleanser containing *Graptophyllum pictum* leaf extract demonstrated antibacterial activity against *A. actinomycetemcomitans*, supporting the broader plausibility of plant-derived antimicrobial strategies in prosthodontic and periodontal contexts [11]. A narrative review of a mucoadhesive gingival patch containing green-tea-derived EGCG similarly concluded that plant-based adjunctive therapies represent a promising, though still preliminary, avenue for periodontal disease management [12]. Nevertheless, despite these encouraging findings, most available evidence regarding natural antibacterial agents against oral pathogens remains fragmented across heterogeneous study designs, preparations, and outcome measures, and rigorous, prospectively registered syntheses spanning both *in vitro* and *in vivo* evidence are lacking. Therefore, this systematic review aimed to evaluate and synthesise the available *in vitro* and *in vivo* evidence on the antibacterial efficacy of bromelain and pineapple-derived extracts against *A. actinomycetemcomitans* and *S. sanguinis*.

MATERIALS AND METHODS

Protocol and Reporting Guideline

This systematic review was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD420261497522, and was conducted and reported in accordance with the PRISMA 2020 Statement [13]. The review protocol specified *a priori* that both *in vitro* and *in vivo* evidence would be sought, reflecting the pre-registered objective of evaluating antibacterial efficacy "across *in vitro* and *in vivo* studies." No amendments were made to the registered protocol during the conduct of the review.

Review Question and Eligibility Criteria

Table 1 presents the review question in PICOS format alongside the criteria applied at each screening stage. No restrictions were imposed based on publication year, language, or study design (randomised and non-randomised experimental studies were both eligible). This approach allowed the inclusion of a directly relevant study published in 2014 and two Indonesian-language reports, which together represented three of the five included studies.

TABLE 1: Review Question and Eligibility Criteria in PICO Format.

PICOS component	Inclusion criteria	Exclusion criteria
Population (P)	<i>Aggregatibacter actinomycetemcomitans</i> and/or <i>Streptococcus sanguinis</i> , investigated in experimental laboratory models, including <i>in vitro</i> and animal studies	Studies testing only non-target microorganisms; studies conducted in human participants
Intervention (I)	Bromelain in any form (purified enzyme, commercial tablet, crude preparation) and extracts derived from any part of the pineapple plant (<i>Ananas comosus</i>), including fruit, peel, core, and hump	Interventions that do not contain bromelain and are not derived from <i>Ananas comosus</i> (pineapple)
Comparator (C)	0.2% chlorhexidine, conventional antibacterial agents or antibiotics, other natural antibacterial agents, vehicle or placebo controls, negative controls, or untreated controls; single-arm studies without a comparator were retained where quantitative outcome data were reported	None applied
Outcome (O)	Measurable antibacterial efficacy expressed as inhibition zone diameter, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), colony-forming unit (CFU) reduction, or biofilm inhibition	Studies reporting no quantitative antibacterial outcome, or reporting outcome data insufficient for extraction
Study design (S)	Randomised and non-randomised experimental studies using <i>in vitro</i> or <i>in vivo</i> (animal) models	Reviews, editorials, letters, conference abstracts without sufficient data, case reports, and case series

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; CFU: colony-forming unit.

Information Sources and Search Strategy

Four databases were searched: PubMed/MEDLINE, Scopus, ProQuest, and Google Scholar, from the inception of each database to 19 July 2026, without restriction on publication date or language, consistent with the registered protocol. Beyond the database searches, the reference lists of all eligible reports were screened to identify additional studies, and supplementary targeted searches in English and Indonesian (covering rat, mouse, and rabbit models) were performed to identify *in vivo* animal studies. The PubMed strategy was built from Boolean operators and free-text keywords combined with the Medical Subject Headings (MeSH) registered in PROSPERO (*Aggregatibacter actinomycetemcomitans*; *Ananas*; Anti-Bacterial Agents; Biofilms; Bromelains; Periodontal Diseases; Periodontitis; *Streptococcus sanguis*): ("bromelain" OR "*Ananas comosus*") AND ("*Aggregatibacter actinomycetemcomitans*" OR "*Streptococcus sanguinis*") AND (antibacterial OR antimicrobial OR antibiofilm OR "*in vivo*" OR animal OR rat OR mice OR rabbit). This string was adapted for each remaining platform, adjusting field tags and syntax to match the indexing conventions of Scopus, ProQuest, and Google Scholar. The animal- and *in vivo*-related terms substantially broadened retrieval, particularly in the large, full-text-indexed ProQuest and Google Scholar platforms, which returned numerous records on unrelated bromelain/pineapple applications (e.g., wound healing, anti-inflammatory, and digestive-health animal studies). In total, 789 records were identified through database searching (ProQuest, n = 369; Google Scholar, n = 320; Scopus, n = 74; PubMed, n = 26); no additional records were retrieved from trial registers.

Study Selection and Data Extraction

Every retrieved record was imported into a reference manager, where duplicates were identified and removed prior to screening. Titles and abstracts were then independently screened by two reviewers (AWN and TS) against the criteria in Table 1. Exclusion criteria were prioritised in the following order, as pre-specified in the PROSPERO protocol: (1) not an eligible experimental *in vitro* or *in vivo* study; (2) intervention does not involve bromelain or a pineapple-derived preparation; (3) does not involve *A. actinomycetemcomitans* and/or *S. sanguinis*; (4) does not report a relevant antibacterial or antibiofilm outcome; and (5) publication type does not provide sufficient eligible data. Reports surviving this pass were retrieved in full and assessed a second time, again independently. Disagreements were resolved through discussion until consensus was reached, and a reason was recorded for every full-text exclusion. Figure 1 traces the entire pathway from identification to inclusion using the PRISMA 2020 flow diagram.

From every included study, the same two reviewers independently used a standardised extraction form to record: study design and experimental setting (*in vitro* or *in vivo*); for *in vivo* studies, the animal species, strain, sex, age or weight, number of animals, and disease model characteristics (not applicable to any included study, as no *in vivo* study met the eligibility criteria); the type and source of bromelain or pineapple-derived preparation, plant part used, extraction or preparation method, concentration or dose, and duration of exposure; the target bacterial species and strain; the comparator or control condition; the assay method; primary outcomes (bacterial growth inhibition, inhibition

zone, MIC, MBC, CFU counts, and bacterial load reduction) and secondary outcomes (biofilm formation, biomass, and reduction); and bibliographic information including funding source and conflict-of-interest declarations. Where a published report left an item unclear or omitted it, that item was recorded as not reported (NR) rather than inferring a value, consistent with the registered data extraction plan.

Risk of Bias Assessment

Methodological quality and risk of bias were assessed independently by two reviewers (AWN and TS) according to study design, as pre-specified in PROSPERO. For *in vitro* studies, the Quality Assessment Tool for *In Vitro* Studies (QUIN) was applied [14], a tool developed and validated through a Delphi process specifically for *in vitro* dental research. Its 12 criteria cover aims, sample size calculation, sampling technique, comparison group, methodology, operator details, randomisation, outcome measurement, outcome assessor, blinding, statistical analysis, and presentation of results. Each criterion was scored as 2 points if adequately specified, 1 if inadequately specified, or 0 if not specified, with not-applicable criteria excluded from the denominator; the final score was calculated as $(\text{total score} \times 100) / (2 \times \text{number of applicable criteria})$, with risk of bias categorised as low ($>70\%$), medium (50–70%), or high ($<50\%$).

For *in vivo* animal studies, SYRCLE's Risk of Bias tool was designated for use [15], an adaptation of the Cochrane Risk of Bias tool for animal intervention studies covering ten items across selection, performance, detection, attrition, reporting, and other sources of bias. As reported in Section 3.6, no *in vivo* study met the eligibility criteria after full-text screening; SYRCLE's tool was therefore not applied, since there were no eligible studies to assess. Any disagreements in risk-of-bias judgements were to be resolved through discussion within the review team.

Data Synthesis

A narrative synthesis was planned for all eligible studies, with findings to be presented according to study type (*in vitro* or *in vivo*), intervention characteristics, target bacteria, and outcome measures, consistent with the registered protocol. Quantitative synthesis and meta-analysis were to be considered where two or more studies were sufficiently homogeneous in study design, intervention, comparator, and outcome measures. In the event, the five included studies diverged substantially in pineapple plant part used, extraction method, degree of bromelain purification, bacterial strains, assay methodology, and outcome

measures, and no meta-analytic model could absorb variation of that breadth without obscuring it; findings were therefore synthesised narratively, grouped by target organism. No subgroup analysis, sensitivity analysis, or formal assessment of publication bias was performed, as none was planned in the absence of a meta-analysis. GRADE was not applied, as that framework was designed for patient-important clinical outcomes, whereas every included study reports laboratory endpoints such as inhibition zones and MIC/MBC values.

RESULTS

Study Selection and Characteristics of the Included Studies

A total of 789 records were identified through database searching (ProQuest, $n = 369$; Google Scholar, $n = 320$; Scopus, $n = 74$; PubMed, $n = 26$); no additional records were retrieved from registers. After removal of 120 duplicate records, 669 records were screened by title and abstract, of which 649 were excluded as clearly irrelevant, the great majority representing bromelain or pineapple-extract studies on unrelated applications (e.g., wound healing, anti-inflammatory activity, digestive health) that had been captured only because of the broadened animal/*in-vivo* search terms. Twenty reports were sought for retrieval, and all were obtained and assessed for eligibility. Fifteen reports were excluded at the full-text stage: irrelevant study design ($n = 3$), irrelevant population ($n = 8$), irrelevant intervention ($n = 2$), and irrelevant publication type ($n = 2$). Consequently, five *in vitro* experimental studies published between 2014 and 2025 met the eligibility criteria and were included in this systematic review (Figure 1); no *in vivo* animal study met the eligibility criteria (Section 3.6). The characteristics of the included studies are summarised in Table 2.

The five studies did not test a common intervention, and this was the dominant source of heterogeneity. Four classes of test material were represented: a commercial bromelain tablet dissolved in dimethyl sulfoxide [16], ethanolic extracts of pineapple core or hump [17,18], hydroethanolic extracts of pineapple peel and fruit flesh tested side by side, which formed the only within-study comparison of plant parts [19], and fresh fruit-flesh supernatant prepared without drying, solvent extraction, or concentration [20]. Susceptibility testing methods were similarly mixed and comprised agar well diffusion [17,19], paper-disc diffusion [20], and broth dilution with turbidimetric reading [16,18], which was confirmed by agar streaking and colony counting in one study [18]. Only one study assessed biofilm formation [17].

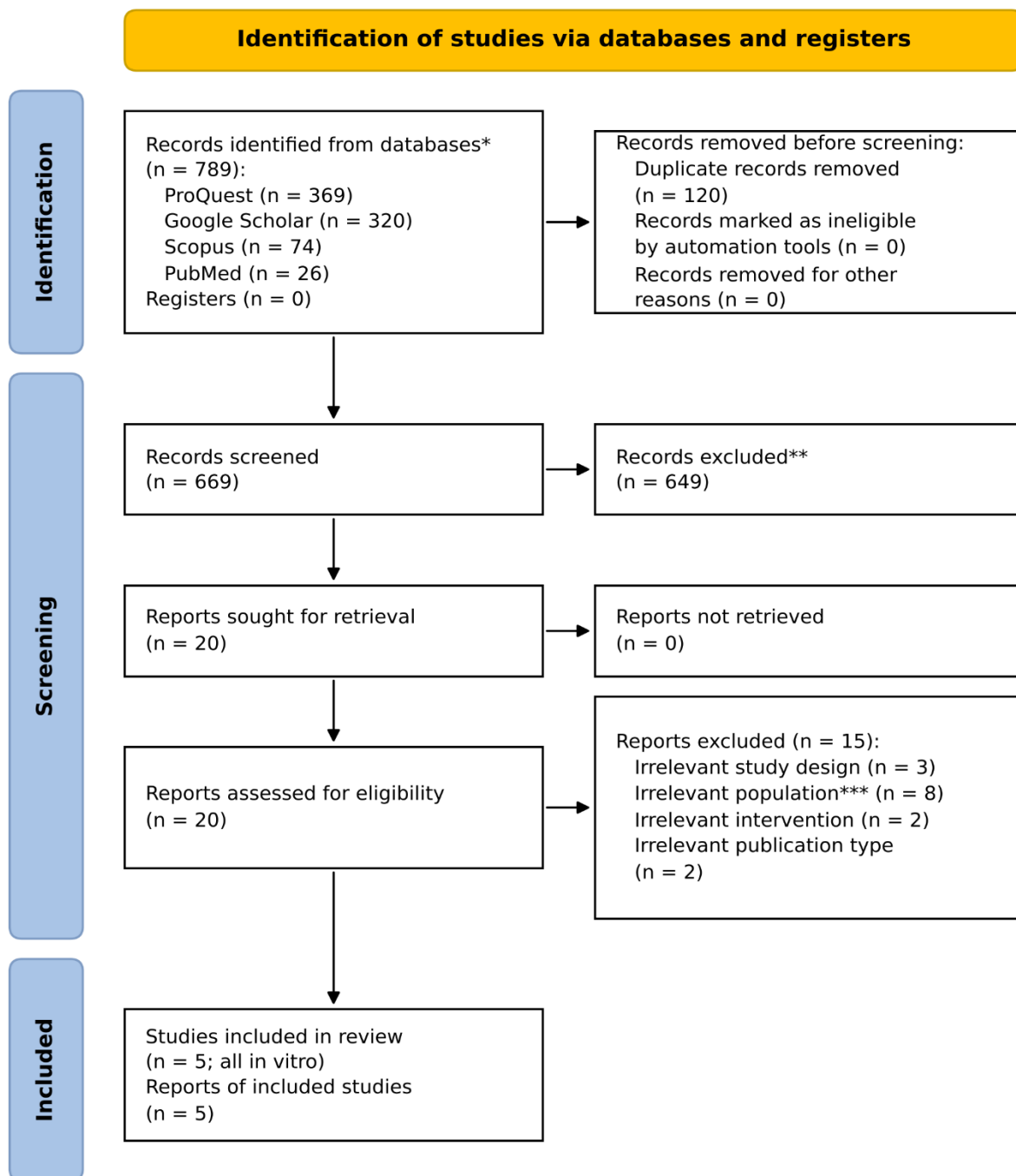


FIGURE 1: PRISMA 2020 Flow Diagram Illustrating the Identification, Screening, Eligibility Assessment, and Inclusion of Studies.

TABLE 2: Characteristics of the Included Studies Evaluating the Antibacterial Efficacy of Bromelain and Pineapple-Derived Extracts Against *A. Actinomycetemcomitans* and *S. Sanguinis*.

Author (Year)	Test material (plant part) and preparation	Tested range	Target bacteria (strain)	Comparator	Assay method and outcome measures	Main findings
Chasanah et al. (2025) [18]	Honey pineapple (<i>Ananas comosus</i> L. Merr) core (bonggol) extract, 96% ethanol maceration	100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78% (two-fold dilutions)	<i>A. actinomycetemcomitans</i> (isolate, Universitas Airlangga; ATCC no. NR)	Growth control and sterile medium control (positive/negative labels applied inconsistently in the original report)	Broth macrodilution (turbidity), confirmed by streaking on solid agar; MIC and MBC (CFU/mL)	MIC = 0.78%; MBC = 6.25%; colony counts fell significantly with increasing concentration (one-way ANOVA, $p < 0.001$)
Goudarzi et al. (2019) [19]	Pineapple fruit flesh and peel, dried and macerated in 85% ethanol for 72 h (hydroethanolic extracts), dissolved in DMSO	1.56–100 mg/mL (two-fold dilutions)	<i>S. sanguis</i> (<i>S. sanguinis</i>) and <i>S. mutans</i> (frozen departmental stock cultures; ATCC no. NR)	<i>Citrus limon</i> peel extract	Agar well diffusion; inhibition zone, MIC, MBC	Peel extract most active against <i>S. sanguis</i> (MIC 1.56 mg/mL; MBC 3.12 mg/mL); fruit extract MIC 12.5 mg/mL against <i>S. sanguis</i> and 25 mg/mL against <i>S. mutans</i>
Praveen et al. (2014) [16]	Commercial bromelain tablets (500 mg) dissolved in DMSO	1–500 mg/mL (ten-step dilution series)	<i>S. mutans</i> (ATCC 25175), <i>E. faecalis</i> (ATCC 35550), <i>A. actinomycetemcomitans</i> (ATCC 29523), <i>P. gingivalis</i> (ATCC 33277)	Not reported	Broth dilution in tube series (described as microdilution), turbidity reading; MIC only	Bromelain inhibited all four species; MIC 2 mg/mL for <i>S. mutans</i> , 4.15 mg/mL for <i>P. gingivalis</i> , 16.6 mg/mL for <i>A. actinomycetemcomitans</i> , and 31.25 mg/mL for <i>E. faecalis</i>
Soulissa et al. (2022) [17]	Pineapple hump (bonggol) extract, 96% ethanol maceration	100, 50, 25, 12.5, 6.25, 3.12%	<i>A. actinomycetemcomitans</i> (ATCC 29522), <i>T. denticola</i> (ATCC 35405), <i>F. nucleatum</i> (ATCC 25586)	0.2% chlorhexidine (positive); distilled water (negative)	Agar well diffusion (inhibition zone) and crystal violet biofilm assay (OD 490 nm) at 1, 6, and 24 h	100% extract inhibited all three species, with efficacy reported as similar to 0.2% chlorhexidine (chlorhexidine zones larger); <i>F. nucleatum</i> also inhibited at 50%; biofilm mass fell in a concentration- and time-dependent manner (ANOVA, post hoc LSD, $p < 0.05$)
Tjiptoningsih and Trisanto (2022) [20]	Crude aqueous extract from fresh pineapple fruit flesh (Honi variety); blended and centrifuged, no solvent extraction	100, 50, 25%	<i>A. actinomycetemcomitans</i> (colonies confirmed on Mueller-Hinton agar; ATCC no. NR)	0.2% chlorhexidine (positive)	Paper-disc diffusion; inhibition zone (caliper, vertical/horizontal diameter)	No inhibition zone (0 mm) at any pineapple concentration; chlorhexidine mean zone 6.08 mm (range 5.77–6.66 mm); independent t-test, $p < 0.001$

All included studies were *in vitro* experimental studies. MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; DMSO: dimethyl sulfoxide; NR: not reported. *S. sanguis* is the former name of *S. sanguinis*.

Risk of Bias within Studies

The results of the QUIN risk-of-bias assessment are presented in Table 3. None of the five included studies achieved a low risk of bias. Four studies were graded as medium risk of bias, with final scores ranging from 54.2% to 58.3% [17–20], while one study was graded as high risk of bias (29.2%) [16]. All five studies clearly stated their aims and objectives (C1) and described an acceptable method of outcome measurement (C8); none provided details of the operator performing the experiments (C6), described a randomisation procedure for the allocation of specimens or plates (C7), identified the outcome assessor (C9), or reported blinding of the assessor to the treatment groups (C10), deficiencies that were universal across the five studies. A sample size was calculated in advance, using the Federer resource-equation formula, in two studies [18,20]; the remaining three gave no sample size justification. The sampling technique (C3) was inadequately specified in all five studies: replicate numbers were stated, but none described how bacterial specimens were selected or allocated to concentration groups. Reporting of the comparison group (C4) was adequate in three studies, which used chlorhexidine or a comparator plant extract [17,19,20]; inadequately specified in one study, whose positive- and negative-control labelling was internally inconsistent [18]; and absent altogether in one study [16]. That same study also gave the least complete account of its methodology (C5) and reported no statistical analysis at all (C11), together explaining its high risk-of-bias rating. A further study stated in its methods that a one-way ANOVA with post hoc Bonferroni correction would be used, but its results section reported only independent t-tests, an inconsistency between planned and reported analysis that reduced its statistical-analysis score [20].

Beyond the QUIN criteria, several limitations relating to the characterisation and reporting of the test materials were identified. None of the included

studies quantified bromelain content or proteolytic activity in the material tested. Dose metrics were also not directly comparable, with three studies reporting concentrations as % v/v of crude extract without specifying dry-matter content and two reporting concentrations in mg/mL, preventing the results from being pooled on a common dose scale. The two lowest reported MIC values, 0.78% in Chasanah et al. [18] and 1.56 mg/mL for peel against *S. sanguinis* in Goudarzi et al. [19], corresponded to the lowest concentrations tested in those studies and should therefore be interpreted as lower-bound observations rather than definitive MIC endpoints. Several internal reporting inconsistencies were also identified, including inconsistent control labels in Chasanah et al., differences between the incubation conditions reported in the abstract and methods of Tjptoningsih and Trisanto, inconsistent inhibition-zone reporting, an MIC of 6.26 mg/mL in Goudarzi et al. that cannot arise from the two-fold dilution series used, inconsistent bacterial identification and mixed units in Praveen et al., and the presentation of inhibition-zone results by Souliissa et al. only as a bar chart without numerical values. In addition, some studies cited earlier studies for their mechanistic rationale; therefore, agreement in the proposed mechanism should not be interpreted as fully independent corroboration, although the experimental data themselves were independent.

Taking together with the heterogeneity of the test materials, these limitations indicate that no individual result in this review is supported by a study assessed as having low risk of bias. The findings should therefore be interpreted cautiously, particularly where conclusions rely on a single study or on MIC/MBC values that were not established across a comparable concentration range. Greater confidence can be placed in findings that are consistent across several studies, such as the anatomical-part gradient and the relative resistance of *A. actinomycetemcomitans*, rather than in any single reported MIC or MBC value.

TABLE 3: Risk-of-Bias Assessment of the Included Studies Using the Quality Assessment Tool for *In Vitro* Studies (Quin).

Study	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	Total	Score (%)	RoB
Chasanah et al. (2025) [18]	2	2	1	1	2	0	0	2	0	0	2	2	14	58.3	Medium
Goudarzi et al. (2019) [19]	2	0	1	2	2	0	0	2	0	0	2	2	13	54.2	Medium
Praveen et al. (2014) [16]	2	0	1	0	1	0	0	2	0	0	0	1	7	29.2	High
Souliissa et al. (2022) [17]	2	0	1	2	2	0	0	2	0	0	2	2	13	54.2	Medium
Tjptoningsih and Trisanto (2022) [20]	2	2	1	2	2	0	0	2	0	0	1	2	14	58.3	Medium

C1: clearly stated aims/objectives; C2: sample size calculation; C3: sampling technique; C4: details of comparison group; C5: detailed methodology; C6: operator details; C7: randomisation; C8: method of outcome measurement; C9: outcome assessor details; C10: blinding; C11: statistical analysis; C12: presentation of results. Scoring: 2 = adequately specified; 1 = inadequately specified; 0 = not specified; NA = not applicable (excluded from the denominator). Final score = (total score × 100) / (2 × number of applicable criteria). Risk of bias (RoB): >70% = low; 50–70% = medium; <50% = high.

Antibacterial Efficacy against

A. actinomycetemcomitans

Overall, the five included studies demonstrated variable antibacterial activity of bromelain and pineapple-derived extracts against *A. actinomycetemcomitans* and *S. sanguinis*. Four studies evaluated *A. actinomycetemcomitans* directly or as one of several target organisms [16–18,20], while one study specifically evaluated *S. sanguinis* alongside *S. mutans* [19]. This distribution reflects the current state of the literature, in which *A. actinomycetemcomitans* has received comparatively more research attention than *S. sanguinis* in the context of bromelain and pineapple-derived antibacterial research.

Among the studies expressing concentrations as a percentage of crude extract, the lowest MIC against *A. actinomycetemcomitans* was obtained with honey pineapple core extract (MIC 0.78%, MBC 6.25%), although this MIC corresponded to the lowest concentration tested and should therefore be regarded as a lower-bound estimate [18]. Bromelain tablets dissolved in dimethyl sulfoxide also showed inhibitory activity against *A. actinomycetemcomitans* at an MIC of 16.6 mg/mL, although this concentration was notably higher than that required to inhibit *P. gingivalis* (4.15 mg/mL) in the same study, suggesting that *A. actinomycetemcomitans* may be comparatively less susceptible to bromelain than other periodontal pathogens [16]. Undiluted (100%) pineapple hump extract inhibited *A. actinomycetemcomitans* with an efficacy that the authors described as similar to that of 0.2% chlorhexidine, although chlorhexidine produced larger inhibition zones, and the same concentration also inhibited *T. denticola* and *F. nucleatum*. The extract further reduced the biofilm mass of all three species in a concentration- and time-dependent manner, with a significant reduction in *A. actinomycetemcomitans* biofilm at concentrations down to 25% after 1 and 6 hours and down to 12.5% after 24 hours [17].

In contrast, crude pineapple fruit extract (Honi variety) produced no measurable inhibition zone against *A. actinomycetemcomitans* at any tested concentration, in direct contrast to the three studies above [20]. This discrepancy may be attributable to methodological differences in the plant part used, the extraction technique, and the resulting bromelain concentration and purity, since core, hump, and purified or tablet preparations consistently outperformed the crude fruit-only extract. The discordant study was also the only one to test fresh, unconcentrated fruit-flesh supernatant by paper-disc diffusion, a format in which a viscous crude preparation and its high-molecular-weight constituents may diffuse poorly into the agar. The original authors also raised this limitation, together with an incubation temperature of 35 °C that may not favour bromelain activity [20]. A null result obtained by disc diffusion alone should therefore not be interpreted as a definitive absence of antibacterial activity without confirmation by broth dilution. However, a genuine absence of antibacterial activity in crude fruit extract cannot be

excluded on the basis of the present evidence, particularly given that the discordant study was itself graded as medium risk of bias and that none of the included studies quantified the bromelain content of the tested preparations.

Antibacterial Efficacy against *S. sanguinis*

Only one included study directly evaluated *S. sanguinis*, comparing hydroethanolic extracts of pineapple fruit and peel against *Citrus limon* peel extract [19]. Pineapple peel extract demonstrated the strongest activity against *S. sanguinis*, with an MIC of 1.56 mg/mL and an MBC of 3.12 mg/mL, more potent than the pineapple fruit extract against the same organism (MIC 12.5 mg/mL, MBC 25 mg/mL) and then the peel extract against *S. mutans* (MIC 6.25 mg/mL, reported as 6.26 mg/mL in the original table). This finding is consistent with the pattern observed in the *A. actinomycetemcomitans* studies above, namely that peel and byproduct-derived pineapple extracts tend to show greater antibacterial potency than crude fruit extracts. A higher concentration of bromelain and of accessory phenolic compounds in the peel has been proposed as a plausible explanation, although none of the included studies measured these constituents, and this mechanism therefore remains speculative.

It should be emphasised that the entire body of evidence concerning *S. sanguinis* rests on a single medium-risk study. Any conclusion regarding the activity of pineapple-derived extracts against this early coloniser is therefore based on unreplicated data and must be regarded as preliminary.

Antibacterial Spectrum and Formulation Dependence

Two studies tested more than one bacterial species simultaneously. Praveen et al. [16] additionally reported bromelain activity against *E. faecalis* and *P. gingivalis*, which, while outside the primary focus of this review, illustrates that its antibacterial spectrum is not restricted to a single periodontal pathogen. Souliissa et al. [17] tested three periodontal pathogens directly relevant to this review, *A. actinomycetemcomitans*, *T. denticola*, and *F. nucleatum*, and found that the undiluted extract inhibited all three, with *F. nucleatum* also inhibited at 50%, indicating that pineapple hump extract's activity is not confined to a single species within this pathogen group. Taken together with the divergent results obtained for different pineapple plant parts, this reinforces a recurring theme across the included studies: the antibacterial efficacy of bromelain and pineapple-derived extracts against *A. actinomycetemcomitans* and *S. sanguinis* is highly formulation-dependent, and conclusions cannot be generalised across all pineapple-based preparations.

In Vivo Evidence

Consistent with the intention to synthesise both *in vitro* and *in vivo* evidence, an extensive search for *in vivo* animal studies was conducted across all four databases together with supplementary, multi-angle targeted searches (in English and Indonesian, spanning rat, mouse, and rabbit models). No study

was identified in which bromelain or a pineapple-derived preparation was tested in an animal model of periodontal disease or oral infection that specifically inoculated, identified, or quantified *A. actinomycetemcomitans* or *S. sanguinis*. Two studies of bromelain in ligature-induced periodontitis in rats were identified during screening but were excluded at the full-text stage because they relied on the animals' natural oral flora without inoculating, identifying, or quantifying either target species, and therefore did not satisfy the population criterion specified in Table 1. As no *in vivo* study met the eligibility criteria, SYRCLE's Risk of Bias tool was not applied, and this absence of eligible *in vivo* evidence is treated as a substantive finding of the review rather than a methodological shortfall (see Section 4.4).

DISCUSSION

Mechanistic Plausibility and Formulation Dependence

Bromelain's antibacterial action is generally attributed to its proteolytic activity against bacterial surface adhesins and extracellular polymeric substances, together with disruption of membrane integrity and interference with quorum-sensing-dependent biofilm maturation [9]. This mechanism plausibly explains why preparations enriched in active enzyme, purified tablets, and extracts derived from the metabolically active core and hump tissue outperformed the crude, water-diluted fruit-flesh extract tested by Tjiptoningsih and Trisanto [20], in which bromelain concentration was neither standardised nor quantified. The same plant-part gradient reappeared in the single *S. sanguinis* study, where peel extract outperformed fruit extract. A similar principle has been applied to red ginger, in which the crude ethanolic extract was fractionated to reduce compositional complexity and improve reproducibility, and the resulting fraction showed low MIC values against *A. actinomycetemcomitans* [4]. Taken together, these observations suggest that the active-compound concentration achieved by a given extraction method, rather than the botanical source per se, is the primary determinant of *in vitro* antibacterial efficacy, a principle with direct implications for how future bromelain-based formulations should be developed and standardised.

Imbalance of Evidence between Target Organisms

A striking asymmetry emerged in the evidence base: four of the five included studies addressed *A. actinomycetemcomitans*, while only one addressed *S. sanguinis*. Because *S. sanguinis* acts as an early biofilm coloniser whose suppression could, in principle, pre-empt the downstream colonisation events that ultimately favour *A. actinomycetemcomitans* establishment [2], this comparative neglect represents a meaningful gap. The single available *S. sanguinis* study reported the lowest MIC (1.56 mg/mL) among the studies expressing concentrations in mg/mL. Because this value also corresponded to the lowest concentration tested, it should be interpreted as a lower-bound estimate, and it strengthens the rationale for future

replication rather than diminishing it. Until independent replication is available, however, any inference regarding bromelain's efficacy against this species should be treated as hypothesis-generating rather than confirmatory.

Quality of the Evidence Base

Every included study carried at least medium risk of bias on QUIN appraisal, and the deficiencies were strikingly uniform: no study reported operator details, a randomisation procedure, outcome-assessor identity, or blinding. These four criteria were not partially met in some studies and absent in others. They were absent across the entire evidence base, indicating a systemic reporting gap in this specific area of *in vitro* dental research rather than an idiosyncrasy of individual research groups. Because none of the studies blinded the person reading a caliper or a turbidity plate to which concentration or extract they were handling, subjective detection bias cannot be excluded in any of the five studies. This is a correctable problem: unlike the absence of *in vivo* evidence, which requires new experimental infrastructure to resolve, blinding and operator reporting are low-cost methodological additions that future *in vitro* studies in this specific research area could adopt immediately.

The Absence of *In Vivo* Evidence as a Substantive Finding

Perhaps the most consequential finding of this review is negative: despite a search strategy explicitly designed, per the PROSPERO protocol, to capture *in vivo* evidence across all species, no eligible *in vivo* study could be located. This absence of evidence is confined to a specific, definable niche, namely the intersection of bromelain/pineapple-derived interventions, an animal model of periodontal or oral infection, and confirmed involvement of *A. actinomycetemcomitans* or *S. sanguinis*. Animal studies of bromelain in ligature-induced periodontitis do exist, but these rely on the rodent's endogenous oral flora rather than deliberately inoculating or quantifying either target species, which is precisely the design choice that would be needed to bridge the gap identified here. Given that comparable mucoadhesive-patch delivery systems have already been validated *in vivo* for other natural agents against periodontal inflammation [7,8], an analogous *in vivo* model, for example, a ligature-induced or *A. actinomycetemcomitans*-inoculated periodontitis model treated with a bromelain-loaded local-delivery system, appears both feasible and a logical next step for this specific research field.

Translational and Clinical Implications

Similar limitations have been highlighted for other natural product-based antimicrobial strategies in dentistry, where promising laboratory findings have not yet been sufficiently validated through *in vivo* studies or randomised clinical trials [5]. The findings of this review reinforce that observation specifically for bromelain and pineapple-derived preparations: while one preparation was described as having an

efficacy similar to that of 0.2% chlorhexidine *in vitro*, none of this evidence has yet been tested under conditions that account for salivary flow, host immune response, or the polymicrobial ecology of the oral cavity. Future research in this area should prioritise (i) standardised and quantified extraction protocols reporting actual bromelain content, so that dose-response relationships can be compared across studies; (ii) blinded and randomised experimental designs with a priori sample size calculation; (iii) vehicle controls wherever dimethyl sulfoxide or ethanol is used, together with heat-inactivated extract controls to distinguish enzymatic from non-enzymatic activity; (iv) evaluation of cytotoxicity and biocompatibility with oral tissues; and (v) a well-designed *in vivo* animal study, assessed against SYRCLE's Risk of Bias tool, as a necessary bridge before well-designed clinical trials in humans can be justified.

Strengths and Limitations

This review has several strengths. It was prospectively registered in PROSPERO, imposed no restriction on publication year or language, which allowed two Indonesian-language reports to be included, and searched explicitly for *in vivo* as well as *in vitro* evidence. Risk of bias was appraised with a tool developed specifically for *in vitro* dental research [14]. Several limitations should also be acknowledged. The evidence base comprised only five studies, and the findings for *S. sanguinis* rest on a single study. No study tested both target organisms, so every comparison between them is indirect. Quantitative pooling was not possible because the studies differed in test material, dose metric, and assay format, and no included study quantified bromelain content. Finally, publication bias could not be formally assessed, and because *in vitro* studies are rarely registered, studies reporting null results may be under-represented.

CONCLUSION

This systematic review demonstrates that bromelain and pineapple-derived extracts have promising but variable antibacterial activity against *A. actinomycetemcomitans* and *S. sanguinis*, with efficacy strongly influenced by the plant part used and the extraction or purification method. Pineapple core, hump, and peel extracts, as well as purified bromelain preparations, generally showed greater activity than crude fruit extracts, although evidence for *S. sanguinis* remains limited. No eligible *in vivo* studies were identified, leaving the available evidence confined to *in vitro* research with medium to high risk of bias. Further standardised and rigorous *in vitro* studies, followed by well-designed animal studies and clinical trials, are needed before bromelain-based products can be recommended for routine periodontal practice.

DECLARATIONS

Conflict of Interest

The authors declare that there is no conflict of interest.

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Ethical Approval

Ethical approval was not required for this study, as it is a systematic review of previously published studies and did not involve human participants or animals.

Data Availability

All data analysed in this systematic review are available within the included studies and the tables of this article.

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