

Review

In-vitro antibacterial activity of propolis extract against periodontopathogenic bacteria: a systematic review



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ABSTRACT

Objectives: The aim of this systematic review was to gather studies that use propolis extract to control antimicrobial activity and evaluate its activity in vitro against major periodontopathogens.

Methods: A search of the PubMed/MEDLINE, Scielo, Lilacs, Science Direct, Cochrane, SCOPUS, Web of Science, and gray literature (Google Scholar and OpenGrey) databases was performed, complemented by a manual search. Articles meeting the following criteria were included: in-vitro studies that evaluated the action of propolis against at least one periodontopathogen.

Results: The database search resulted in 1473 articles. After analysis of the titles and abstracts, 179 articles were duplicated, and 18 were selected for full-text screening, of which five were excluded. The manual search resulted in one article. This systematic review comprised 13 articles. *Porphyromonas gingivalis* appeared in all studies, and propolis showed an antibacterial effect, demonstrating the minimum inhibitory concentration.

Conclusions: The propolis extract shows an effective antimicrobial activity on the periodontal pathogenic bacteria and may be a viable therapeutical option for the modulation of periodontitis. More studies are important to analyze the cytotoxic effects of different concentrations on gingival fibroblasts. (Rev Port Estomatol Med Dent Cir Maxilofac. 2024;65(1):3-14)

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Atividade antibacteriana in vitro do extrato de própolis contra bactérias periodontopatogénicas: uma revisão sistemática

R E S U M O

Palavras-chave:

Doença periodontal
Porphyromonas gingivalis
 Própolis
 Revisão sistemática

Objetivos: O objetivo desta revisão sistemática foi reunir estudos que utilizam o extrato de própolis para o controlo da atividade antimicrobiana e avaliar sua atividade in vitro contra os principais periodontopatógenos.

Métodos: Foi realizada uma busca nas bases de dados PubMed/MEDLINE, Scielo, Lilacs, Science Direct, Cochrane, SCOPUS, Web of Science e na literatura cinzenta (Google Scholar and OpenGrey), complementada por uma busca manual, e foram incluídos artigos que atenderam aos critérios: estudos in vitro que avaliaram a ação da própolis contra pelo menos um periodontopatógeno.

Resultados: A busca na base de dados resultou em 1473 artigos. Após análise dos títulos e resumos, 179 artigos estavam duplicados, 18 artigos foram selecionados para triagem do texto completo e cinco deles foram excluídos. A busca manual resultou em um artigo. Esta revisão sistemática compreendeu 13 artigos. A *Porphyromonas gingivalis* apareceu em todos os estudos e a própolis apresentou efeito antibacteriano demonstrando a partir da concentração inibitória mínima.

Conclusões: O extrato de própolis apresenta atividade antimicrobiana efetiva sobre as bactérias patogénicas periodontais, podendo ser uma opção terapêutica viável para a modulação da periodontite. Mais estudos são importantes para analisar os efeitos citotóxicos de diferentes concentrações nos fibroblastos gengivais. (Rev Port Estomatol Med Dent Cir Maxilofac. 2022;65(1):3-14)

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Introduction

Periodontal disease is a biofilm-induced chronic inflammatory disorder.¹⁻³ Its primary cause is the bacterial biofilm that triggers an inflammatory response, causing irreversible damage to periodontal tissues and consequent tooth loss if untreated.^{4,5} Thus, one of the aims of periodontal treatment is to reduce the number of pathogenic microorganisms in contact with periodontal tissues,² including orange and red complex pathogens such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*.³

Non-surgical periodontal treatment is based on mechanical debridement,⁶ which is combined with subgingival irrigation to eliminate bacteria and improve its outcome.^{4,7,8} Periodontology is particularly concerned with using natural compounds, and propolis extract is an example found in solutions for subgingival irrigation. In-vitro studies have shown the susceptibility of periodontopathogenic microorganisms to solutions prepared from propolis extract.⁸

Propolis is the generic name for a type of glue, a complex resinous material that bees collect from the buds and exudates of plants to construct and repair honeycombs.⁴ Several biological activities have been attributed to propolis, including antifungal, antibiotic, antiprotozoal, antiviral, antitumor, and anti-inflammatory properties.⁵ The main chemical classes in propolis are flavonoids and phenolic and aromatic compounds, while volatile oils, terpenes, and bee wax do not significantly contribute to its properties or chemical effects.^{4,5,9-11}

Despite the increasing use of propolis worldwide, few studies have evaluated the inhibitory activity of propolis against important dental pathogenic anaerobic bacteria, such as periodontopathogenic bacteria.⁹⁻¹¹ The objective of this systematic review was to gather studies that used propolis extract to control antimicrobial activity and evaluate its in-vitro activity against major periodontopathogens.

Material and Methods

This report complies with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.¹² The protocol was not registered, given the nature of the investigated population.

A search was performed on the PubMed/MEDLINE, Scielo, Lilacs, Science Direct, Cochrane, SCOPUS, and Web of Science databases, complemented by a manual search. We also searched the gray literature in Google Scholar and OpenGrey using appropriate strategies; only the first 100 articles of the Google Scholar search were analyzed. We searched for published studies evaluating the antimicrobial activity of propolis extract against at least one type of periodontopathogenic bacteria, using other substances already known as a comparison. There were no language and time restrictions. The search was updated in January 2023. The search strategies are described in Table 1.

The eligibility criteria were as follows: in-vitro studies evaluating the action of propolis against *Porphyromonas gingivalis*;

Table 1. Search strategy used for the electronic databases.

Database	Search Strategy
Medline through PubMed (http://www.ncbi.nlm.nih.gov/pubmed)	("propolis"[MeSH Terms] OR "propolis"[All Fields] OR ("flavonoidal"[All Fields] OR "flavonoide"[All Fields] OR "flavonoides"[All Fields] OR "flavonoidic"[All Fields] OR "flavonoids"[MeSH Terms] OR "flavonoids"[All Fields] OR "flavonoid"[All Fields]) OR ("flavonoids"[MeSH Terms] OR "flavonoids"[All Fields] OR "bioflavonoid"[All Fields] OR "bioflavonoids"[All Fields])) AND ("periodontal"[All Fields] OR "periodontally"[All Fields] OR "periodontically"[All Fields] OR "periodontics"[MeSH Terms] OR "periodontics"[All Fields] OR "periodontic"[All Fields] OR "periodontitis"[MeSH Terms] OR "periodontitis"[All Fields] OR "periodontitides"[All Fields] OR ("periodontal diseases"[MeSH Terms] OR ("periodontal"[All Fields] AND "diseases"[All Fields]) OR "periodontal diseases"[All Fields] OR ("periodontal"[All Fields] AND "disease"[All Fields]) OR "periodontal disease"[All Fields]) OR ("porphyromonas gingivalis"[MeSH Terms] OR "porphyromonas"[All Fields] AND "gingivalis"[All Fields]) OR "porphyromonas gingivalis"[All Fields] OR ("periodontal"[All Fields] OR "periodontally"[All Fields] OR "periodontically"[All Fields] OR "periodontics"[MeSH Terms] OR "periodontics"[All Fields] OR "periodontic"[All Fields] OR "periodontitis"[MeSH Terms] OR "periodontitis"[All Fields] OR "periodontitides"[All Fields]) AND ("therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "treatments"[All Fields] OR "therapy"[MeSH Subheading] OR "therapy"[All Fields] OR "treatment"[All Fields] OR "treatment s"[All Fields])) AND (("anti infective agents"[Pharmacological Action] OR "anti infective agents"[MeSH Terms] OR ("anti infective"[All Fields] AND "agents"[All Fields]) OR "anti infective agents"[All Fields] OR "antimicrobial"[All Fields] OR "antimicrobials"[All Fields] OR "antimicrobially"[All Fields]) AND ("activable"[All Fields] OR "activate"[All Fields] OR "activated"[All Fields] OR "activates"[All Fields] OR "activating"[All Fields] OR "activation"[All Fields] OR "activations"[All Fields] OR "activator"[All Fields] OR "activator s"[All Fields] OR "activators"[All Fields] OR "active"[All Fields] OR "acted"[All Fields] OR "actively"[All Fields] OR "actives"[All Fields] OR "activities"[All Fields] OR "activity s"[All Fields] OR "activities"[All Fields] OR "motor activity"[MeSH Terms] OR ("motor"[All Fields] AND "activity"[All Fields]) OR "motor activity"[All Fields] OR "activity"[All Fields]))
Science Direct (https://www.sciencedirect.com/), OpenGrey (http://www.opengrey.eu/)	(propolis OR flavonoids OR bioflavonoids) AND (periodontitis OR periodontal disease OR porphyromonas gingivalis OR periodontal treatment) AND (antimicrobial activity)
Scopus (http://www.scopus.com/)	TITLE-ABS-KEY ((propolis OR flavonoids OR bioflavonoids) AND (periodontitis OR periodontal AND disease OR porphyromonas AND gingivalis OR periodontal AND treatment) AND (antimicrobial AND activity))
Web of Science (http://webofknowledge.com/)	((propolis OR flavonoids OR bioflavonoids) AND (periodontitis OR periodontal disease OR porphyromonas gingivalis OR periodontal treatment) AND (antimicrobial activity))
Scielo (www.scielo.org/)	(propolis OR flavonoids OR bioflavonoids) AND (periodontitis OR periodontal disease OR porphyromonas gingivalis OR periodontal treatment) AND (antimicrobial activity)
Cochrane (https://www.cochranelibrary.com/)	(propolis OR flavonoids OR bioflavonoids) AND (periodontitis OR periodontal disease OR porphyromonas gingivalis OR periodontal treatment) AND (antimicrobial activity) in Title Abstract Keyword
Lilacs (http://lilacs.bvsalud.org/)	(propolis OR flavonoids OR bioflavonoids) [Palavras] and (periodontitis OR periodontal disease OR porphyromonas gingivalis OR periodontal treatment) [Palavras] and (antimicrobial activity) [Palavras]

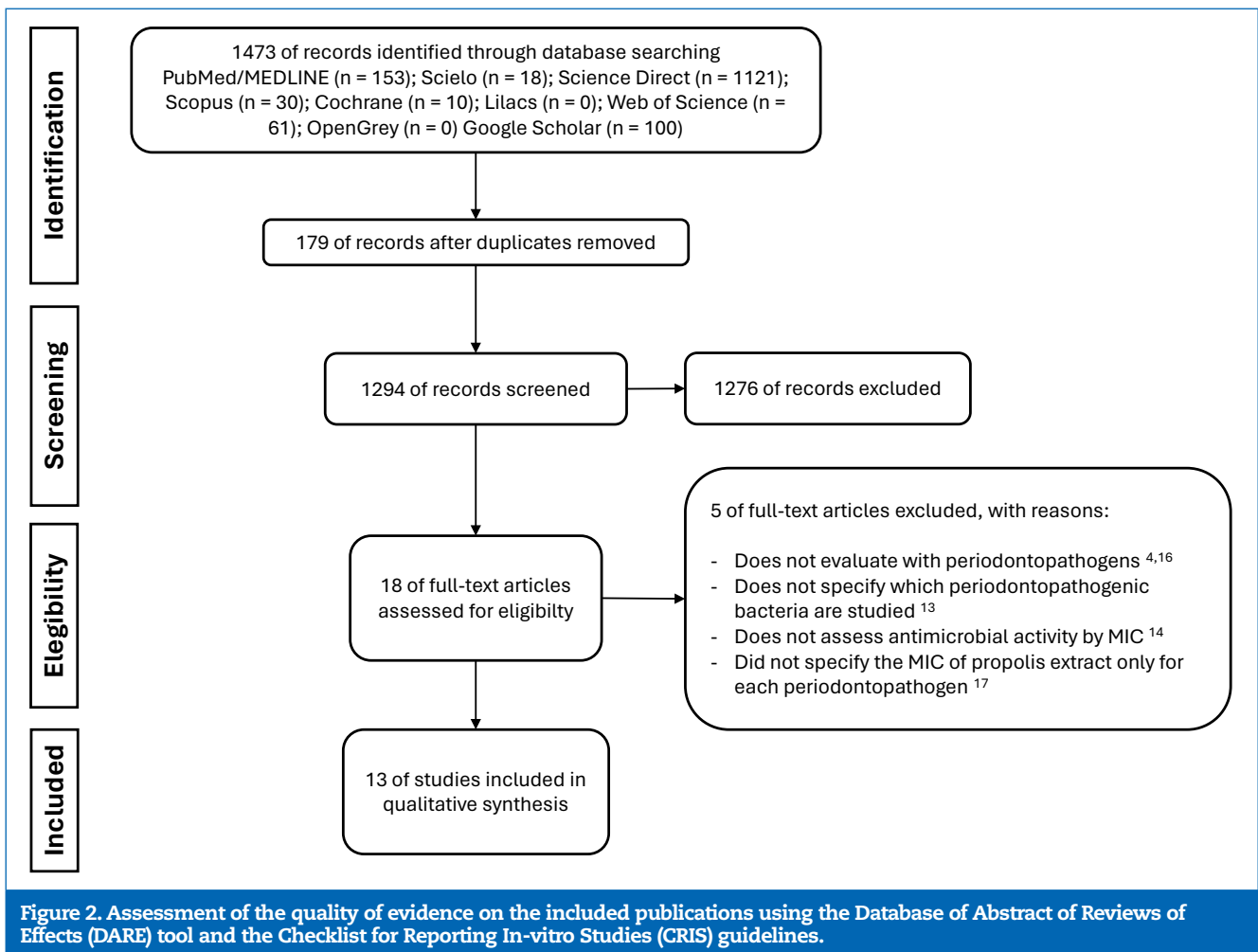
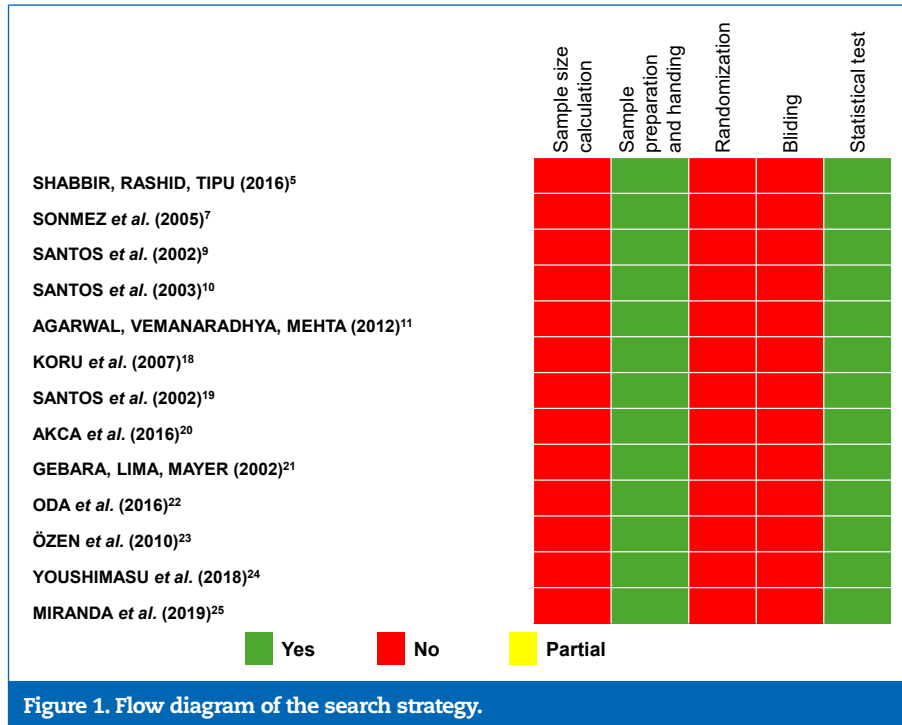
MeSH – Medical Subject Heading.

studies evaluating the antimicrobial activity of propolis using the minimum inhibitory concentration (MIC); studies whose full text was available. The exclusion criteria were in-vivo studies, case reports, case series, literature reviews, and systematic reviews. There was no language restriction for the search and inclusion of articles.

Two researchers (J. G. A. M. and D. M. S.) independently preselected the studies using the eligibility criteria to analyze the titles and abstracts. We first performed a calibration exercise with 20% of the retrieved studies, which confirmed the reviewers' ability to analyze the remaining titles and abstracts to preselect articles. Then, they read the full text of the studies preselected, and those that met the eligibility criteria were included in the review. In case of disagreement between the two researchers, a third reviewer (S. C. S.) reevaluated the articles for possible inclusion in the study.

The following data were extracted from the included studies: year of publication, author(s), origin of the propolis samples, method of propolis extraction, oral bacterial strains used, and evaluation of antimicrobial activity based on MIC.

The quality of evidence on the included publications was assessed using the Database of Abstract of Reviews of Effects (DARE) tool¹³ and Checklist for Reporting In-vitro Studies (CRIS) guidelines.¹⁴ Five questions were considered: (i) Was the sample size calculation included?; (ii) Were sample preparation and handling adequate?; (iii) Were the samples randomized?; (iv) Was there blinding?; (v) Was a correct statistical test used? The interpretation for scoring "yes," "partially," and "no" is described in Figure 1. Two independent researchers (J. G. A. M. and D. M. S.) performed the quality assessment process and tried to resolve any dispute via discussion. If necessary, a third reviewer (S. C. S.) was consulted to reach a consensus.



The risk of bias in the studies included was appraised using the QUIN method for in-vitro studies.¹⁵ Two reviewers (D. M. S. and J. G. A. M.) evaluated the QUIN tool's 12 criteria. Each criterion was then assigned a score: 2 for "adequately specified," 1 for "inadequately specified," and 0 for "not specified." Criteria recognized as not applicable were not included in the final score. The studies' risk of bias was considered low when the final score was above 70%, medium between 50% and 70%, and high below 50%.

Results

The database search resulted in the retrieval of 1473 articles. After analysis of the titles and abstracts, 179 duplicated articles were excluded, and 18 articles were selected for full-text screening, of which five were then excluded.^{4,14-17} Thus, this systematic review comprised 13 articles (Figure 2).^{5,7,9-11,18-25}

The studies evaluated the antimicrobial activity of pure propolis against *Porphyromonas gingivalis* and other periodontopathogenic bacteria (*Prevotella intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*). They analyzed propolis collected in different regions of Brazil and the world, as well as the main oral bacterial strains. Table 2 summarizes the propolis samples' region of origin, periodontal bacterial strains, and positive and negative controls used in the microbiological studies.

As indicated in Table 2, the crude propolis samples were diluted in ethanol to different concentrations, and the extracts were obtained using different techniques. The MIC was the lowest extract concentration to inhibit the visible growth of the test organism. The agar diffusion test was used in all studies to describe the MIC of antimicrobial activity.

All 13 articles included evaluated the action of propolis extract against *P. gingivalis* and other periodontopathogenic bacteria, including *P. intermedia*,^{5,7,19,21,23} *F. Nucleatum*,^{7,10,18,21} *A. Actinomycetemcomitans*,^{10,11,20,22,25} and *P. nigrescens*.^{19,24} One of the articles also evaluated the action of propolis extract against the growth of *C. Albicans*.²

Five of the articles included used clinical use antibiotics, such as ampicillin,²⁴ amoxicillin with clavulanic acid,⁵ clindamycin,¹⁹ tetracycline,^{5,10,19,24} meropenem,^{9,10,19} penicillin,^{9,19} metronidazole,^{5,19} and erythromycin,¹⁹ as comparison groups regarding propolis extract against the aforementioned microorganisms. Articles by Shabbir et al.⁵ and Santos et al.¹⁹ reported the tested bacteria showed resistance to tetracycline^{5,19} and clindamycin,¹⁹ but were susceptible to propolis extract. In contrast, Santos et al.¹⁰ observed that all bacteria analyzed were susceptible to the antibiotics used (tetracycline and meropenem) and propolis extract.

Chlorhexidine, at 0.12%²⁵ and 0.2% concentrations,²⁰ was also used as a comparison. Miranda et al.²⁵ found that propolis extract significantly reduced orange complex species, while chlorhexidine was less effective. The same authors reported that both propolis extract and chlorhexidine significantly reduced the proportions of red complex specimens associated with periodontal disease. Akca et al.²⁰ suggested that propolis extract would be more effective against *P. intermedia* than chlorhexidine.

In the quality of evidence assessment, no study indicated whether the sample calculation was performed or whether there was a randomization or blinding process for the MIC analysis. However, all studies explained how sample preparation and handling were performed and whether a correct statistical test was used (Figure 2). Table 3 presents, in detail, the results of the risk of bias assessment of the studies included in this systematic review. All articles included presented a moderate risk of bias.

Discussion

The present systematic review evaluated studies that used propolis to inhibit the microbial activity of oral bacteria. Several antimicrobial agents are used as adjuvants to mechanical therapy in periodontal disease. The choice of propolis was based on the increasing attention given to natural products.⁸ Among the adjuvant therapies tested, propolis has shown antimicrobial properties, which justifies the investigation of this phytotherapeutic agent against periodontopathogenic bacteria.

In Santos et al. study,⁹ *P. anaerobius*, *P. gingivalis*, and *P. intermedia* were the bacteria most susceptible to propolis, with MICs ranging from 128 to 256 µg/ml. Meropenem and penicillin G were used as positive controls, and their MIC ranged from 0.03 to 8 and 4 µg/ml, respectively. Compared to these values, the MICs of the ethanolic extract of propolis and its fractions do not appear to be significant. However, the antibacterial activity of propolis is highly significant since many of the bacteria tested are resistant to clinically used antibiotics.⁹

The systemic administration of antimicrobial agents can lead to multidrug-resistant microorganisms, transfer of resistance determinants, and side effects. Several studies have demonstrated the resistance of bacterial strains to tetracycline. In turn, clinical isolates resistant to tetracycline were found to be susceptible to propolis extract. One factor that may explain the lack of development of bacterial resistance to propolis is the wide variety of active agents in its composition associated with the need for synergism among these compounds.^{5,9,10,18,19,26} These essential characteristics permit propolis to act against diverse oral pathologies, reducing the prescription of antimicrobials and consequent bacterial resistance.

Brazilian green propolis with some isolated compounds, such as artepillin C, baccharin, and ursolic acid, causes a depolarization of the *P. gingivalis* membrane, making it more permeable.²³ Yoshimasu et al.²⁴ concluded that the combined therapy of Brazilian green propolis ethanolic extract with low doses of antibiotics would increase antibiotics' effectiveness, but such synergistic activity should be further studied. The use of the Brazilian green propolis ethanolic extract with the main antibiotics in addition to periodontal treatment is, therefore, an interesting practice to reduce the adverse effects caused by antibiotics and their time of administration.

Ethanol extraction is the most popular method for the production of propolis extracts.²⁷ Park et al.²⁸ concluded that the 80% ethanol extract resulted in the largest quantity of flavonoids and that most flavonoids identified were extracted

Table 2. Propolis samples used in the studies, techniques used for propolis extraction, bacterial culture, minimum inhibitory concentration (MIC) of propolis extract, and the main results reported in the studies evaluated.

Study	Funding	Origin of propolis	Extraction of propolis	Bacterial strains	Control	Bacterial culture	MIC	Main results
SHABBIT, RASHID, TIPU (2016) ⁵	University of Health Sciences, Lahore, Pakistan,	Propolis from Skardu and Islamabad (Pakistan)	Addition of 95% ethanol.	<i>P. saccharolytica</i> ; <i>P. gingivalis</i> ; <i>P. intermedia</i> ; <i>Prevotella melaninogenica</i>	Amoxicillin-clavulanic acid; tetracycline; metronidazole	For each experiment, the bacteria were inoculated on anaerobic basal agar supplemented with 5% defibrinated horse blood incubated at 35°C in anaerobic jars for five days. After five days, the same colonies were also plated on blood agar and incubated aerobically at 37°C to confirm their anaerobic nature.	0.064-0.512 mg/ml	Propolis from Pakistan exerted potent antimicrobial activity against <i>P. gingivalis</i> .
Sonmez et al. (2005) ⁷	No funding	Propolis from Turkey, Germany (Sigma), Australia, and United States	Some samples were dissolved in 70% ethanol, others in propylene glycol; 10% Sigma propolis in 70% ethanol; 10% Sigma propolis in propylene glycol; 10% Turkish propolis in 70% ethanol; 10% Turkish propolis in propylene glycol; Australian propolis, non-alcoholic 30% liquid; North American propolis containing 20% water-soluble extract.	<i>P. gingivalis</i> ; <i>P. intermedia</i> ; <i>Campylobacter recto</i> ; <i>Fusobacterium nucleatum</i> ; <i>Candida albicans</i> ; <i>Candida parapsilosis</i> ; <i>Candida krusei</i>	Agar plates without solutions	Bacteria were cultured on anaerobic blood agar, thioglycolate broth, and cooked meat broth. After 48 h, the turbidity of bacterial suspensions was adjusted to 0.5 McFarland turbidity standard, and they were diluted by 1/10 ratio.	1/128-1/256 mg/ml	The dilutions of propolis samples were effective in inhibiting periodontopathogens.
Santos et al. (2002) ⁹	No funding	Propolis from Minas Gerais	Dissolved in 80% ethanol (3 x 70 ml) at 70°C for 30 min, followed by chromatography on a silica gel column and separation between immiscible solvents.	<i>A.a.</i> ; <i>Fusobacterium nucleatum</i> ; <i>Fusobacterium necrophorum</i> ; <i>P. gingivalis</i> ; <i>P. intermedia</i> ; <i>P. nigrescens</i> ; <i>Eubacterium lentum</i> ; <i>Peptostreptococcus anaerobius</i>	Meropenem; penicillin G	Bacteria were inoculated in Brucella agar supplemented with 0.5% yeast extract, hemine (5 mg/ml), menadione (1 mg/ml) and 5% horse blood. Incubation was performed at 37°C, in anaerobic conditions, for 5 days in Brewer-like anaerobic jars (90% N ₂ , 5% CO ₂ and 5% H ₂).	128-256 µg/ml	The antimicrobial activity of propolis is relevant and may represent an alternative for the treatment of periodontopathogens.
Santos et al. (2003) ¹⁰	National Council for Scientific and Technological Development (CNPq) and Research Support Foundation of the State of Minas Gerais	Propolis collected during the dry and rainy season in Minas Gerais	Preparation at 25% in ethanol.	<i>A.a.</i> ; <i>Fusobacterium spp.</i> ; <i>P. intermedia</i> ; <i>P. nigrescens</i> ; <i>P. gingivalis</i>	Meropenem; tetracycline	<i>A. a.</i> strains were inoculated in tryptic soy agar, supplemented with 0.5% yeast extract and 5% sheep blood. <i>Fusobacterium spp.</i> strains were inoculated in BHI agar supplemented with 0.5% yeast extract, L-cysteine at 0.075%, and 5% sheep blood. All strains were incubated at 37°C. Anaerobiosis (90% CO ₂ , 5% N ₂ , and 5% H ₂) was used for <i>Fusobacterium</i> and black-pigmented anaerobes, and microaerophilia for <i>A. a.</i> in Brewer-like anaerobiosis jars.	0.025-0.05%	The concentrations of the ethanolic extract of propolis necessary to exert antimicrobial activity seem to be high compared to commonly used antimicrobial agents.

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Table 2. Propolis samples used in the studies, techniques used for propolis extraction, bacterial culture, minimum inhibitory concentration (MIC) of propolis extract, and the main results reported in the studies evaluated.

Study	Funding	Origin of propolis	Extraction of propolis	Bacterial strains	Control	Bacterial culture	MIC	Main results
Agarwal, Vemanaradhya, Mehta (2012) ¹¹	No funding	Chinese	Addition of 95% ethanol. The solution obtained was filtered and adjusted by adding 80% ethanol.	A.a.; <i>P. gingivalis</i>	Dimethyl sulfoxide	Nutrient broth was used to obtain the viable growth of bacteria from their freeze-dried form. After 48 h, turbidity in the test tube confirmed the growth of bacteria, which was compared and adjusted to McFarland 0.5 turbidity standard (108 colony-forming units per milliliter). Petri dishes containing 100 ml of BHI broth supplemented with 5 ml of 5% sheep blood were inoculated with approximately 100 µl of the respective microbial strain using the swab technique.	<i>P.g.*</i> 0.1-0.0125 µg/ml; <i>A.a.*</i> 0.1-0.025 µg/ml	The concentrations inhibited oral pathogens but were also cytotoxic to gingival fibroblasts.
Koru et al. (2007) ¹⁸		Propolis from Anatolia (Turkey) and Brazil	The concentrated solution obtained by dilution of propolis in ethanol (1:10) was evaporated to dryness. About 5 mg of the residue was mixed with 75 ml dry pyridine and 50 ml bis(trimethylsilyl) trifluoroacetamide (BSTFA) heated to 80°C for 20 min.	<i>Peptostreptococcus anaerobius</i> ; <i>Peptostreptococcus micros</i> ; <i>Prevotella oralis</i> ; <i>Prevotella melaninogenica</i> ; <i>P. gingivalis</i> ; <i>Fusobacterium nucleatum</i> ; <i>Veillonella parvula</i> ; <i>Lactobacillus acidophilus</i> ; <i>Actinomyces naeslundii</i>	Agar plates without ethanolic propolis extract; agar plates containing 1% alcohol (final concentration)	The bacteria were inoculated into 5% blood BHI agar, supplemented with menadione (1 mg/mL) and hemin (5 mg/mL), and incubated under anaerobic conditions at 37°C (an anaerobic jar with a gas-generating kit) for 48-56 h.	4-512 µg/ml	The Turkish propolis samples were more active against anaerobic oral pathogens than the Brazilian propolis sample.
Santos et al. (2002) ¹⁹		Propolis from Minas Gerais	The ethanolic extract of propolis was standardized by submitting them to a temperature of 50°C, after which the resin content was diluted with 95% ethanol to obtain stock solutions of 100 mg/mL.	<i>P. intermedia</i> ; <i>P. nigrescens</i> ; <i>P. gingivalis</i>	Meropenem; penicillin G; tetracycline; clindamycin; metronidazole; erythromycin	The bacteria were inoculated in Brucella agar supplemented with 0.5% yeast extract, hemin (5 mg/mL), menadione (1 mg/mL), 5% horse blood and incubated at 37°C in anaerobiosis for 5 days in Brewer-like anaerobiosis jars (90% N ₂ , 5% CO ₂ and 5% H ₂).	<i>Pi.*</i> 128 µg/ml; <i>Pn.*</i> 256 µg/ml; <i>Pg.*</i> 128 µg/ml	The antimicrobial activity of propolis is relevant and may represent an alternative for the treatment of periodontopathogens.
Akca et al. (2016) ²⁰	No funding	Propolis from Turkey	Dissolved in 80% ethanol.	<i>S. mutans</i> ; <i>S. sobrinus</i> ; <i>L. acidophilus</i> ; <i>L. salivarius</i> subsp. <i>salivarius</i> ; <i>E. faecalis</i> ; <i>S. aureus</i> ; <i>A.a.</i> ; <i>A. israelii</i> ; <i>P. gingivalis</i> ; <i>P. intermedia</i> ; <i>C. albicans</i>	Agar plates without the propolis solutions and with chlorhexidine and 80% ethanol	The aerobic bacteria were cultured in 5 mL of BHI broth at 37°C for 48 hours in the microaerophilic atmosphere with 5% CO ₂ . The anaerobic bacteria were cultured in autoclaved-sterilized fastidious anaerobe broth supplemented by sheep blood (50 mL/L), vit. K (1 µg/mL), and hemin (5 µg/mL), at 37°C for 6-7 days in an anaerobic chamber in an atmosphere consisting of 90% N ₂ , 5% CO ₂ , and 5% H ₂ .	64-1,024 µg/ml	The propolis extract exhibited bactericidal activity against <i>L. acidophilus</i> , <i>L. salivarius</i> subsp. <i>salivarius</i> and <i>P. intermedia</i> ; the strongest bactericidal effect was observed for <i>C. albicans</i> and <i>E. faecalis</i> ; the lowest effect compared to chlorhexidine was found for <i>S. aureus</i> , <i>A. actinomycetemcomitans</i> and <i>E. faecalis</i> .

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Table 2. Propolis samples used in the studies, techniques used for propolis extraction, bacterial culture, minimum inhibitory concentration (MIC) of propolis extract, and the main results reported in the studies evaluated.

Study	Funding	Origin of propolis	Extraction of propolis	Bacterial strains	Control	Bacterial culture	MIC	Main results
GEBARA, LIMA, MAYER (2002) ²¹	No funding	Propolis from Brazil	Dilution in 70% ethanol.	<i>P. intermedia</i> ; <i>Prevotella melaninogenica</i> ; <i>P. gingivalis</i> ; <i>A.a.</i> ; <i>Capnocytophaga gingivalis</i> ; <i>Fusobacterium nucleatum</i> ; <i>Canidada albicans</i> ; <i>Pseudomonas aeruginosa</i> ; <i>Escherichia coli</i> ; <i>Staphylococcus aureus</i>	Plates with serial ethanol concentrations	The bacteria were cultured in enriched brain heart infusion agar for 5 days. The resultant cultures were diluted in phosphate buffer solution to reach concentrations equivalent to McFarland scale n° 1.	0.25-1.0 µg/ml	The propolis extract exhibited in-vitro antimicrobial activity against <i>F. nucleatum</i> , <i>P. gingivalis</i> , <i>P. intermedia</i> , <i>P. melaninogenica</i> , <i>A. actinomycetemcomitans</i> , and <i>C. gingivalis</i> .
ODA et al. (2016) ²²		Brazilian green propolis	Extracted with ethanol water and dissolved in ethanol. The final ethanol concentration was 0.1%.	<i>S. mutans</i> ; <i>S. sanguinis</i> ; <i>A.a.</i> ; <i>P. gingivalis</i>	Penicillin G; streptomycin	<i>S. mutans</i> was cultured in BHI medium under aerobic conditions at 37°C in an incubator. <i>S. sanguinis</i> and <i>A. a.</i> were cultured in BHI medium at 37°C in a CO ₂ gas incubator (5% CO ₂ , 95% air). <i>P. gingivalis</i> was cultured in BHI medium containing 4 µg/ml hemin and 0.4 µg/ml menadione at 37°C in an anaerobic incubation chamber (N ₂ : 80%, CO ₂ : 10%, H ₂ : 10%).	50-2,000 µg/ml	Brazilian green propolis inhibited the growth of <i>P. gingivalis</i> , <i>S. mutans</i> and <i>S. sanguinis</i> , but not of <i>A.a.</i>
ÖZEN et al. (2010) ²³	No funding	Propolis from Turkey	The concentrated solution of propolis extract was evaporated to dryness, and 5 mg of the residue was mixed with 75 µl dry pyridine and 50 ml BSTFA heated to 80°C.	<i>Peptostreptococcus magnus</i> ; <i>Eubacterium lentum</i> ; <i>Lactobacillus acidophilus</i> ; <i>Actinomyces odontolyticus</i> ; <i>P. intermedia</i> ; <i>P. oralis</i> ; <i>P. melaninogenica</i> ; <i>P. gingivalis</i> ; <i>Fusobacterium nucleatum</i> ; <i>Bliphilia wadsworthia</i> ; <i>Veillonella parvula</i>	Agar plates with 1% ethanol; agar plates containing the culture medium plus ethanol	The bacteria were inoculated in Brucella agar plates supplemented with 0.5% yeast extract, hemine (5 µg/ml), menadione (1 µg/ml) and 5% horse blood. Incubation was performed at 37°C under anaerobic conditions for 5 days in an anaerobic jar with a gas-generating kit.	0.4-0.6 mg/ml	Antimicrobial activity was higher against anaerobic bacteria.
YOUSHIMASU et al. (2018) ²⁴	No funding	Brazilian green propolis collected during the rainy season in Minas Gerais	The crude propolis was pulverized with a homogenizer and extracted in ethanol. After stirring at room temperature for 12 hours, the filtration was evaporated until the solid content reached 55% to yield ethanol-extracted propolis (EEP). EEP was standardized to contain a minimum of 8.0% artepillin C. EEP was diluted to 1% with ethanol (vehicle control) prior to use in the present study.	<i>P. gingivalis</i> , <i>P. nigrescens</i> , <i>Fusobacterium nucleatum</i> , <i>A.a.</i> , <i>Prevotella bescarii</i> , <i>Streptococcus anginosus</i> , <i>Streptococcus cristatus</i> , <i>Streptococcus gordoni</i> , <i>Streptococcus mitis</i> , <i>Streptococcus mutans</i> , <i>Streptococcus salivarius</i> , <i>Streptococcus oralis</i> , <i>Streptococcus sanguinis</i> , <i>Streptococcus sobrinus</i> , <i>Escherichia coli</i>	Agar plates containing 1% ethanol (vehicle control); ampicillin; tetracycline	The bacteria were anaerobically grown in BHI broth supplemented with hemin and menadione (HM) at concentrations of 5 and 1 µg/mL, respectively, or on BHI-HM blood agar plates (BAP). BHI broth and BHI BAP that did not include HM were used to maintain the other periodontopathic bacterial species (<i>Fusobacterium nucleatum</i> and <i>A.a.</i>) and commensal oral streptococci. All strains were grown in an anaerobic chamber in 80% N ₂ , 10% H ₂ , and 10% CO ₂ at 37°C.	<i>P.g.</i> * 64 µg/ml (broth); 256 µg/ml (agar) <i>P.n.</i> * 128 µg/ml (broth and agar) <i>F.n.</i> * >128 µg/ml (broth); 1024 µg/ml (agar) <i>A.a.</i> * >128 µg/ml (broth); 2048 µg/ml (agar)	Results suggest prophylactic and therapeutic potential for ethanol-extracted propolis as a membrane-acting antibacterial agent against periodontal diseases.

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(continuation)

Table 2. Propolis samples used in the studies, techniques used for propolis extraction, bacterial culture, minimum inhibitory concentration (MIC) of propolis extract, and the main results reported in the studies evaluated.

Study	Funding	Origin of propolis	Extraction of propolis	Bacterial strains	Control	Bacterial culture	MIC	Main results
MIRANDA et al. (2019) ²⁵	Coordination for the Improvement of Higher Education Personnel (CAPES); National Council for Scientific and Technological Development (CNPq) and Institute Foundation for the development of the Amazon (FIDESA)	Brazilian red propolis from Maceio	The BRP ethanolic extract was obtained by adding 25 g of propolis into 200 ml of 80% ethanol under continuous mixing for 45 min. Next, the suspension was filtered using a paper filter, the solvent was evaporated using rotary evaporator equipment, and the BRP ethanolic extract was obtained with a yield of 73%.	Actinomyces naeslundii, Actinomyces oris, Actinomyces gerencseriae, Actinomyces israelii, Veillonella parvula, Actinomyces odontolyticus, Streptococcus sanguinis, Streptococcus oralis, Streptococcus intermedius, Streptococcus gordoni, Streptococcus mitis, A. a., Capnocytophaga ochracea, Capnocytophaga gingivalis, Capnocytophaga gingivalis, Capnocytophaga sputigena, Streptococcus constellatus, Eubacterium nodatum, Fusobacterium nucleatum vitcentii, Parvimonas micra, Fusobacterium nucleatum polymorphum, Campylobacter showae, Fusobacterium periodonticum, P. intermedia, P. gingivalis, Tannerella forsythia, Eubacterium saburreum, Streptococcus anginosus, Selenomonas noxia, Propionibacterium acnes, G. morbillorum, S. mutans	Dilution vehicle - 6.4% ethanol in 10% phosphate buffer (negative control) and 0.12% chlorhexidine (positive control)	Most strains (e.g., Actinomyces subsp., Streptococcus subsp., and Fusobacterium subsp.) were grown on tryptone soy agar with 5% sheep blood under anaerobic conditions (85% nitrogen, 10% carbon dioxide, and 5% hydrogen), while Eubacterium subsp. and N. mucosa were cultured on fastidious anaerobic agar with 5% ram blood. P. gingivalis and P. melaninogenica were grown on tryptone soy agar containing yeast extract enriched with 1% hemin, 5% menadione, and 5% sheep blood. T. forsythia was grown on tryptone soy agar containing yeast extract enriched with 1% hemin, 5% menadione, 5% sheep blood, and 1% N-acetylglucosamine. After 24 h, the bacterial inocula were transferred to glass tubes with BHI supplemented with 1% hemin and allowed to grow for 24 h.	P.g., P.i., F.n.* 1.600 µg/ml ⁻¹	Brazilian red propolis (BRP) extract was as effective as chlorhexidine in reducing overall subgingival biofilm formation and was better than chlorhexidine in reducing orange-complex bacterial populations in an in-vitro subgingival multispecies biofilm model.

*P.g.: Porphyromonas gingivalis; A.a.: Aggregatibacter actinomycetemcomitans; P.i.: Prevotella intermedia; P.a.: Prevotella nigrescens; F.n.: Fusobacterium nucleatum. BHI: brain heart infusion.

Table 3. Risk of bias of studies included in the systematic review using the QUIN tool.

Criteria	Shabbir, Rashid, Tipu (2016) ⁵	Sonmez et al. (2005) ⁷	Santos et al. (2002) ⁹	Santos et al. (2003) ¹⁰	Agarwal, Vemanaradiya, MEHTA (2012) ¹¹	Koru et al. (2007) ¹⁸	Santos et al. (2002) ¹⁹	Akca et al. (2016) ²⁰	Gebara, Lima, Mayer (2002) ²¹	Oda et al. (2016) ²²	Özen et al. (2010) ²³	Youshimasu et al. (2018) ²⁴	Miranda et al. (2019) ²⁵
Clearly stated aims/objectives	2	2	2	2	2	2	2	2	2	2	2	2	2
Detailed explanation of sample size calculation	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Detailed explanation of sampling technique	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Details of comparison group	2	2	2	2	2	2	2	2	2	2	2	2	2
Detailed explanation of methodology	2	2	2	2	2	2	2	2	2	2	2	2	2
Operator details	0	0	0	0	0	0	0	0	0	0	0	0	0
Randomization	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Method of measurement of outcome	2	2	2	2	2	2	2	2	2	2	2	2	2
Outcome assessor details	0	0	0	0	0	0	0	0	0	0	0	0	0
Blinding	0	0	0	0	0	0	0	0	0	0	0	0	0
Statistical analysis	2	2	2	2	2	2	2	2	2	2	2	2	2
Presentation of results	2	2	2	2	2	2	2	2	2	2	2	2	2
Final score	66.6	66.6	66.6	66.6	66.6	66.6	66.6	66.6	66.6	66.6	66.6	66.6	66.6
Estimated risk of bias	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium

2: adequately specified; 1: inadequately specified; 0: not specified; NA: not applicable

with 60 to 80%. Furthermore, the inhibition of microbial growth was considerably higher at this concentration. Based on these studies, it would be interesting to standardize the concentration of ethanol for propolis extraction in order to obtain adequate antimicrobial activity for propolis collected in any region.

Propolis extract can be obtained using different techniques. The traditional method is maceration, which is slow, requiring 2 to 10 days.^{29,30} Extraction by ultrasound is faster and more efficient.³¹ It is important to establish the best technique for extracting samples collected in different regions to obtain the highest antimicrobial activity.

The subgingival microbiota is dominated by Gram-negative rod-shaped bacteria.³² Koru et al.¹⁸ observed that the MICs of anaerobic Gram-positive bacteria were lower than those of anaerobic Gram-negative bacteria; thus, the latter were more sensitive to the ethanolic extract of propolis. In contrast, in the studies of Akca et al.²⁰ and Grange and Davey,³³ the results of biofilm analysis did not confirm that finding, as propolis was more effective against Gram-positive bacteria. Koru et al.¹⁸ also found that all anaerobic bacterial strains were susceptible to the ethanolic extract of propolis, regardless of its region of origin.

Oda et al.²² demonstrated a weak inhibitory effect of Brazilian green propolis on *A. actinomycetemcomitans*. Bacterial growth was observed up to the highest concentration of 2,000 µg/ml, the dose limit that does not cause cytotoxic effects on gingival fibroblasts. Sonmez et al.⁷ found that Australian propolis (non-alcoholic, 30%) and North American propolis (alcoholic, 20%) effectively inhibited antimicrobial activity, but the concentrations tested were cytotoxic to gingival fibroblasts. Agarwal et al.¹¹ evaluated Chinese propolis, and the concentrations that inhibited oral pathogens, including *P. gingivalis*, were also cytotoxic to gingival fibroblasts.

The studies by Miranda et al.²⁵ and Figueiredo et al.²⁶ show that the ethanolic extract of Brazilian red propolis has a significant effect on multispecies biofilms of pathogens associated with periodontal disease and can be a great ally when associated with non-surgical periodontal treatment.

The bacteria used in experimental studies can be derived from clinical isolates or reference strains. Most papers included in this study used reference strains.^{7,9,11,18,20-24} None of the included articles assessed only clinical isolate strains, but the studies by Santos et al.⁹, Santos et al.,¹⁰ and Shabbir et al.⁵ used part of bacterial strains from clinical isolates and part of reference strains.

It is understood that periodontal diseases result from a "dysbiotic" biofilm and not directly from the effect of specific pathogenic bacteria on the host. However, some key pathogens, such as *Porphyromonas gingivalis*, play an important role in the imbalance with the host, increasing the pathogenicity of the bacteria in the biofilm and inducing a disease state.³⁴ This understanding of the polymicrobial dependence of a dysbiotic biofilm for the emergence of periodontal diseases indicates the need to carry out more experimental studies that use a microbial environment similar to those found in individuals with periodontal disease. Using isolated strains may not configure an evaluation of the periodontal pathological condition close to the real one.

One of this study's limitations is that the quality of evidence of the studies included in this systematic review was not assessed using GRADE because in-vitro studies do not generate evidence with immediate clinical application. Besides, several points analyzed by the GRADE tool cannot be applied in a methodology of experimental studies in vitro.

Conclusions

In conclusion, the results show that propolis extract exhibits effective antimicrobial activity against the main oral bacteria and might be a feasible therapeutic option for the modulation of periodontitis, in addition to reducing the use of antibiotic therapy for oral pathogens and consequently the risk of bacterial resistance. Further studies are important to better understand the mechanisms of propolis' anti-inflammatory and antimicrobial actions and analyze the cytotoxic effects of different concentrations on gingival fibroblasts.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

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