ARTICULO DE INVESTIGACIÓN

EFECTO ANTIMICROBIANO DEL PROPÓLEO SOBRE *Streptococcus mutans:* REVISIÓN SISTEMÁTICA DE LA LITERATURA

ANTIMICROBIAL EFFECT OF PROPOLIS ON Streptococcus mutans: SYSTEMATIC REVIEW OF THE LITERATURE

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RESUMEN

Para controlar la caries dental, se emplean diversas terapias; sin embargo, surgen alternativas de productos naturales con propiedades que inhiben el desarrollo de caries producidas por bacterias, como es el caso del propóleo, producto de la abeja. **Objetivo:** determinar el efecto antimicrobiano del propóleo sobre la bacteria *Streptococcus mutans* mediante una revisión sistemática de la literatura científica publicada entre 2019 - marzo de 2024 en revistas indexadas. **Aspectos Metodológicos:** Se empleó el método Prisma y el tamaño muestral empleado fueron 43 artículos seleccionados en las bases de datos y revistas indexadas, considerando criterios de inclusión y exclusión previamente establecidos. **Resultados:** El propóleo difiere en sus propiedades en función de la región de procedencia; las concentraciones mínimas inhibitorias se ubican en distintos rangos para diferentes tipos de propóleo; este producto ha demostrado tener efecto

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antimicrobiano contra diversas bacterias, incluyendo *Streptococcus mutans*, gracias a sus más de 300 sustancias activas identificadas, las cuales interfieren con la actividad enzimática necesaria para el crecimiento bacteriano. Productos comerciales dedicados a la salud dental incluyen compuestos del propóleo para el tratamiento de microorganismos, incluidas las bacterias. **Conclusiones:** El propóleo, aunque posee una estructura biológica y química en relación directa con la región donde se obtiene, muestra actividad antimicrobiana contra *Streptococcus mutans*. Sus componentes activos pueden inhibir el crecimiento de esta bacteria, interferir con su adhesión a los dientes y reducir la producción de ácidos que dañan el esmalte. Se requiere continuar las investigaciones y ensayos clínicos para determinar de manera precisa su eficacia frente a *S. mutans*.

PALABRAS CLAVE: Propóleo, Efecto antimicrobiano, *Streptococcus mutans*.

ABSTRACT

To control dental caries, various therapies are used; However, alternatives are emerging for natural products with properties that inhibit the development of cavities caused by bacteria, such as propolis, a bee product. **Objective:** Determine the antimicrobial effect of propolis on the *Streptococcus mutans* bacteria through a systematic review of the scientific literature published between 2019 - March 2024 in indexed journals. **Methodological aspects:** The Prisma method was used and the sample size used was 43 articles selected from the databases and indexed journals, considering previously established inclusion and exclusion criteria. **Results:** Propolis differs in its properties depending on the region of origin; minimum inhibitory concentrations are located in different ranges for different types of propolis; this product has been shown to have an antimicrobial effect against various bacteria, including *Streptococcus mutans*, thanks to its more than 300 identified active substances, which interfere with the enzymatic activity necessary for bacterial growth; commercial products dedicated to dental health include propolis compounds for the treatment of microorganisms, including bacteria. **Conclusions:** propolis, although it has a biological and chemical structure in direct relation to the region where it is obtained, shows antimicrobial activity against *Streptococcus mutans*. Its active components can inhibit the growth of this bacteria, interfere with its adhesion to teeth and reduce the production of acids that damage enamel. Continued research and clinical trials are required to precisely determine its effectiveness against *S. mutans*.

KEYWORDS : Propolis, Antimicrobial effect, *Streptococcus mutans*.

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INTRODUCTION

Dental caries is a multifactorial oral pathology involving interactions among various genetic, microbial, behavioral, social, and environmental factors. Streptococcus mutans is one of the main acidogenic bacteria responsible for the development of cariogenesis (Asgharpou et al., 2019). S. mutans is a facultative Gram-positive bacterium capable of fermenting a wide range of sugars; the organic acids it excretes cause a localized drop in pH, which can lead to lesions in the dental enamel and thus initiate the development of caries (Djais et al., 2019).

Currently, research into natural products that can inhibit the development of caries caused by bacteria is being promoted, such as propolis, which due to its low toxicity, has gained attention for the prevention and treatment of oral diseases. The chemical composition of propolis extracts is complex and varies depending on the region and time of collection, due to variations in the flora from which pharmacologically active substances are obtained. It is therefore essential to verify their antimicrobial activity (Navarro Pérez et al., 2021).

The antimicrobial effect of propolis occurs at two levels: first, through direct action on the microorganism, and second, by stimulating the immune system, which activates the body's natural defenses. It has been shown that the antimicrobial activity of propolis is greater against Gram-positive bacteria than Gram-negative ones, making it a good candidate to inhibit S. mutans (Przybyłek and Karpiński, 2019). In this regard, the aim of this work is to determine the antimicrobial effect of propolis on Streptococcus mutans through a systematic literature review.

METHODOLOGY

This research adopted the format of a systematic review of existing scientific literature on propolis and its effect on the bacterium *Streptococcus mutans*. The sample consisted of 43 articles selected from databases and indexed journals available through Universidad Antonio Nariño, referencing the antimicrobial effect of propolis on S. mutans, and obtained via search engines like PubMed, Google Scholar, SciELO, and Science Direct.

Inclusion criteria: (a) articles focused on the antimicrobial effect of propolis on S. mutans, (b) written in English or Spanish, (c) published in indexed journals between 2019 and March 2024. Exclusion criteria: (a) duplicate or incomplete articles in databases, (b) monographs, (c) articles available only as abstracts.

The systematic review followed the PRISMA method (Preferred Reporting Items for Systematic Reviews and Meta-

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Analyses), an internationally recognized guideline for conducting and reporting systematic reviews and meta-analyses (Rethlefsen et al., 2021). The process involved: **Identification**: of relevant studies with key descriptors from January 2019 to March 2024, **selection**: of studies based on predefined eligibility criteria, **data extraction**: of relevant information from the selected studies, **risk of bias assessment**: reviewing adherence to exclusion criteria, **data synthesis**: summarizing the extracted data, **interpretation and presentation of results**: summarizing and interpreting the overall antimicrobial effect of propolis on S. mutans, **dissemination**: of the systematic review process, protocols, and results in the final report.

RESULTS AND DISCUSSION

Antibacterial Effect of Different Types of Propolis Depending on Geographical Origin on S. mutans

Propolis has a variety of biological properties due to its complex chemical composition, which is directly related to the plant biodiversity of its production area (Ribeiro et al., 2021). As a substance derived from botanical compounds collected by bees, there are different types (green, brown, and red) depending on chemical composition and geographical origin (Martins et al., 2019; Almuhayawi, 2020).

Middle Eastern propolis has shown the highest antibacterial efficacy (Almuhayawi, 2020). Tunisian propolis, especially from the Béja region, may be efficiently used as raw material in the food and pharmaceutical industries due to its phenolic richness and potential health benefits (Gargouri et al., 2019). Lower activity was observed in samples from Germany, Ireland, and Korea (Przybyłek & Karpiński, 2019). Chinese propolis essential oil also exhibits antibacterial activity against S. mutans by inhibiting cell viability within biofilms, reducing total biofilm biomass, and destroying biofilm structure (Yuan et al., 2022). Some European propolis types contain flavonoids and phenolic acids that could serve as markers for standardizing and evaluating the quality of propolis and its preparations (Wieczorek et al., 2022). Spanish propolis is effective against Streptococcus oralis, S. mutans, and S. sanguinis, which are dominant species in dental plaque (Navarro Pérez et al., 2021).

Carvalho et al. (2019) found both Brazilian green and red propolis to be effective against multiple oral pathologies. A study by Veloz et al. (2019) demonstrated that Chilean propolis has antibiofilm properties against S. mutans, with Chilean polyphenols showing antimicrobial activity at low concentrations and reducing or inhibiting biofilm proliferation. Ethanolic extract of Peruvian propolis showed that S. mutans was sensitive (+) and very sensitive (++) based on Duraffourd's scale (Collatupa and Tito,

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2021). Brazilian brown propolis demonstrated high chemical complexity in its volatile fraction and bactericidal activity (Ribeiro et al., 2021). Brazilian red propolis showed antibacterial activity and could be a potential agent to reduce cariogenic biofilm buildup.

In an *in vitro* essay, a red propolis ethanolic extract exhibited acceptable cytotoxicity and reduced colonization of S. mutans (Martins et al., 2019). No studies were found related to the use of Colombian-origin propolis as an antibacterial agent.

It is concluded that the antibacterial effect of different types of propolis on S. mutans can vary depending on their geographical origin, and that propolis from certain regions may be more effective than others due to their specific content of active compounds. The authors suggest specific research to determine the antibacterial effect of particular types of propolis on S. mutans based on their geographical origin.

Minimum Inhibitory Concentration of Propolis on S. mutans

An assay to evaluate the antimicrobial activity of Spanish ethanolic propolis extract showed that S. sanguinis was more sensitive, with its growth being inhibited at lower concentrations than those required for S. mutans. The estimated minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for S. mutans were 240 μg/mL (0.4%) and 480 μg/mL (0.8%), respectively. MICs to eradicate S. mutans growth were also reported for propolis from other regions: Argentina – 50 μg/mL, Brazil – 293 μg/mL, and Poland – 39–156 μg/mL, among others (Navarro Pérez et al., 2021).

According to Collatupa and Tito (2021), a chemically characterized ethanolic extract of Peruvian propolis showed antibacterial activity against S. mutans. The average inhibition halos of the four experimental groups demonstrated that the 25% concentration showed the highest antibacterial activity, with halos measuring 17.582 mm, although all four concentrations generated well-defined inhibition zones.

A study by Neelima et al. (2020) showed that glass ionomer cement (GIC) is known for its antimicrobial activity due to its low pH and fluoride release. The experiment added propolis, chitosan, and chlorhexidine for greater microbial efficacy against S. mutans. The average inhibition zone (in mm) against S. mutans for groups I, II, III, and IV were 11.70 ± 1.49, 16.50 ± 2.23, 19.30 ± 2.87, and 15.60 ± 2.76, respectively. It was concluded that propolis and chlorhexidine were effective in inhibiting *S. mutans.*

A test conducted by Veloz et al. (2019) to determine the inhibitory concentration using the serial dilution method in soy broth with sucrose and different concentrations of polyphenol-rich

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propolis extract showed that propolis reduces biofilm formation and metabolic activity in S. mutans biofilms at all concentrations tested. In another study, Tambur et al. (2021) prepared serial dilutions of essential oils in plates. Test plates contained 100, 50, 25, and 12.5 µg/mL of active essential oils. Propolis dilutions were 50, 25, 12.5, and 6.3 µg/mL of active solutions. Results showed that propolis solutions dissolved in benzene, diethyl ether, and methyl chloride demonstrated equal efficacy against all tested strains at 12.5 µg/mL and were effective against S. mutans.

An in vitro study by Saputra et al. (2020) used adhesive samples, where the control group was modified with glass ionomer cement support. Two groups were supplemented with 15% and 25% propolis for 15 and 30 days to evaluate the antibacterial effect of each sample against *S. mutans* using an agar diffusion test.

The results showed that a 25% concentration of propolis inhibited S. mutans growth more effectively than the 15% concentration and the group without propolis (control group). Another experimental laboratory study used alcoholic propolis and calculated the minimum inhibitory concentration (MIC) for four bacterial species, including S. mutans, using the agar dilution method. The results indicated that the propolis mouthwash was more effective against the oral bacteria studied compared to other rinses (Nazery et al., 2019).

An in vitro evaluation comparing the antibacterial activity of ethanolic green tea extract with 10% and 20% ethanolic propolis extract concluded that the 20% propolis extract had greater antibacterial activity against Streptococcus mutans strains (ATCC 25175) than the green tea extract (Cayo Rojas and Ganoza, 2020).

A study that quantified the volatile compounds of Brazilian brown propolis and evaluated their biological activities found that the oil had an antibacterial effect by inhibiting the growth of Streptococcus mutans at concentrations of 25 µg/mL and 50 µg/mL (Porto et al., 2021).

The minimum inhibitory concentration (MIC) of propolis against Streptococcus mutans may vary, although it generally ranges from 12.5 µg/mL to 480 µg/mL, as derived from the reviewed articles. This variation depends on various factors such as bacterial strain, bacterial growth, propolis composition, and testing conditions (method used, concentration, substrate, duration). Overall, MIC values fall within different ranges for various types of propolis, and their comparison is complicated by the use of different measurement units (%, µg/mL).

It is important to note that the exact concentration may require specific laboratory testing for precise determination. Table 1 presents the conversion from µg/mL to mg/L of the minimum inhibitory concentrations reported by each author.

Table 1 Conversion from µg/mL to mg/L of the minimum inhibitory concentrations of the investigated of each author.Minimum Inhibitory Concentration.

Authors	Minimum Inhibitory Concentration
(Navarro Pérez et al., 2021).	240 µg/mL (0,24 mg/L), 480 µg/mL (0,48 mg/L), 50 µg/mL (0,05 mg/L), 293 µg/mL (0,293 mg/L), 39–156 µg/mL (0,039- 0,156 mg/L)
(Tambur et al., 2021)	100, 50, 25 y 12,5 µg/mL (0,1, 0,05, 0,025, 0,0125 mg/L), 50, 25, 12,5, 6,3 µg/mL (0,05, 0,025, 0,0125, 0,0063 mg/L)
(Porto et al., 2021)	25 µg/mL (0,025 mg/L), 50 µg/mL (0,05)

Relationship Between the Active Compounds in Propolis and Its Antibacterial Effect on S. mutans

More than 300 compounds have been identified in propolis, including phenolic compounds (flavonoids, phenolic acids, and esters), aromatic acids, essential oils, waxes, and amino acids. Propolis collected in Egypt is known to contain poplar components, as well as caffeic acid esters and long-chain fatty alcohols such as tetradecanol, hexadecanol, and dodecanol. Russian propolis contains flavonols and flavones. Likewise, the main source of Brazilian propolis is resin, which includes components such as diterpenes, lignans, prenylated derivatives of p-coumaric acid, acetophenone derivatives, and flavonoids.

Brazilian propolis contains artepillin C and caffeic acid phenethyl ester. Some compounds are found only in tropical areas, such as sesquiterpenoids, including germacrene D, ledol, and spatulenol. One type of Cuban propolis contains benzophenyl and polyisoprenylated compounds (Anjum et al., 2019).

Otreba et al. (2022) confirm the presence of various active substances in propolis and highlight the antibacterial activity, particularly of flavonoids, which increase bacterial membrane permeability and inhibit bacteria by affecting genetic coding, nucleic acid synthesis, adhesion and biofilm formation, energy production, bacterial metabolism, and bacterial nucleic acid synthesis. Phenolic compounds are among the most common bioactive ingredients in propolis and possess anti-inflammatory, antimicrobial, and antioxidant properties (Moghadam et al., 2020). According to Veloz et al. (2019), the antimicrobial agents in propolis produce changes in biofilm structure and cell aggregation due to fluctuations in protein levels and enzymatic expression.

In the study by Djais et al. (2019), the ethanolic extract of propolis did not show inhibitory effects on S. mutans biofilm formation. They hypothesized that the extracellular matrix may have interfered with the antimicrobial properties of the propolis tested.

Tambur et al. (2021) demonstrated a positive inhibitory effect of different propolis solutions and essential oils on the growth of oral microorganisms, representing an alternative preventive and therapeutic approach for individuals at high risk of dental caries and other oral diseases.

The ethanolic extract of Spanish propolis contains mainly terpenes, diterpenes, and terpenoid compounds (Collatupa & Tito, 2021). Caffeic acid phenethyl ester (a polyphenol) is the most abundant compound in Chilean propolis. The effect of polyphenols on S. mutans occurs through a combination of mechanisms to reduce bacterial growth and affect biofilm formation or proliferation by altering its architecture. Pinocembrin and apigenin, two bioactive flavonoid compounds, also have strong antimicrobial and antibiofilm activity (Veloz et al., 2019). Flavonoids show antimicrobial effects by inhibiting virulence factors, efflux pumps, biofilm formation, membrane disruption, cell wall synthesis, nucleic acid synthesis, acid synthesis, and bacterial motility (Biharee et al., 2020).

The results reveal high contents of total phenolics, flavonoids, and polyphenols. Ethyl ester, galangin, and genistein were the main identified compounds. Flavonoids are organic compounds characterized by a variety of phenolic structures, which give them antibacterial properties, as shown by clinical and in vitro studies (Hasnat et al., 2024). Farnesol, a sesquiterpene found in propolis and citrus fruits, shows promising antibacterial activity for the treatment and prevention of caries, but its hydrophobicity limits clinical use (Yi et al., 2020).

The combination of active compounds in propolis—such as flavonoids, caffeic acid, fatty alcohols, diterpenes, artepillin C, germacrene D, benzophenyl, and polyisoprenylated compounds (Anjum et al., 2019)—supports its antibacterial effect on S. mutans and may benefit oral health by preventing dental caries.

Propolis-Based Products for Oral Health

To combat diseases, including oral diseases, natural environments have been explored for their healing properties. Their use represents greater availability, lower costs, and fewer side effects (Moghadam et al., 2020). Carvalho et al. (2019) note that these elements generate lower toxicity, greater biocompatibility, and better pharmacological activity. Martins et al. (2019) explain that natural products exhibit lower bacterial tolerance, lower toxicity, and no taste changes. Researchers are dedicating time, effort, and resources to discover bioactive compounds and find applications for oral health (Moghadam et al., 2020).

Anjum et al. (2019) state that propolis has a wide range of pharmacological potential with antibacterial applications, and when used as an individual component, it may act synergistically, leading to diverse biological effects. As a complement, the ethanolic extract of propolis used in mouthwashes and toothpaste improves microbial prevention, treats infections, and is effective in managing gum inflammation.

Propolis is commercially available in toothpaste, lozenges, mouthwashes, creams, gels, chewables, tablets, and antiseptic blends (Anjum et al., 2019). Although not yet common as an active ingredient in American toothpastes, it is emerging due to its anti-inflammatory, immunomodulatory, antioxidant, antimicrobial, and antidiabetic properties (Saeed et al., 2021).

According to Martins et al. (2019), the red propolis extract of Spanish origin was effective against biofilm even at low concentrations, suggesting its use in the development of non-toxic complementary products for the control of dental caries.

It is necessary to properly identify the compounds responsible for the biological activity of propolis to facilitate the development of standardized preparations, ensuring higher quality and efficacy. Moreover, propolis could be considered a source for isolating biologically active substances (Martins et al., 2019).

Commercial products dedicated to dental health include propolis compounds for treating microorganisms, including bacteria (antibacterials). Its use is also being explored in combination with other products as a therapeutic supplement in dentistry. In conclusion, in oral health, propolis is used in various products due to its antibacterial and anti-inflammatory properties.

CONCLUSIONS

The effectiveness of propolis varies depending on its concentration and quality, which may be influenced by its geographical origin, as this determines its chemical composition and biological activity. Mediterranean propolis, especially that from Tunisia, shows high antibacterial activity due to its phenolic acid and diterpene content. In contrast, Chinese propolis demonstrates greater antibacterial action against Gram-

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positive bacteria (Streptococcus mutans), while the lowest antibacterial activity has been observed in propolis from Germany, Ireland, and Korea.

Spanish propolis requires higher concentrations to be effective against Streptococcus mutans compared to other oral bacteria, though it could be used as a marker for standardization and quality assessment. In the Americas, Brazilian red, green, and brown propolis has shown effectiveness against conditions affecting the oral cavity.

Red propolis demonstrated the greatest antibacterial activity. In Chile, it has been effective at low concentrations, while Peruvian propolis, according to the Duraffourd Scale, showed significant antibacterial activity at all concentrations tested.

The minimum inhibitory concentration of propolis against Streptococcus mutans can vary, generally ranging from 12.5 μg/mL to 480 μg/mL, depending on several factors such as the bacterial strain, strain growth, propolis composition, and testing conditions (method used, concentration, substrate, time).

Propolis has shown antimicrobial effects against various bacteria, including Streptococcus mutans, thanks to more than 300 identified active substances, which interfere with the enzymatic activity of several proteins essential for bacterial growth and development. In conclusion, the active compounds in propolis are beneficial for the treatment of bacterial infections in the oral cavity, specifically in the prevention of dental caries and inhibition of plaque formation.

The use of natural products like propolis for preventing oral diseases has increased due to its cost advantages, availability, and fewer side effects compared to conventional products. Commercial products that include propolis compounds—such as toothpastes, lozenges, mouthwashes, creams, gels, chewables, and tablets—used individually or in combination, form a therapeutic supplement due to their antibacterial properties.

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