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*Short report*

# Evaluation of the antimicrobial activity of *Olea europaea* (Olive) against cariogenic bacterium *Streptococcus mutans*

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**Abstract:** The use of plants as a medicinal source has increased enormously in the last three decades due to important advantages such as safety, efficacy and global availability. Dental caries is the most prevalent chronic bacterial disease in people worldwide; the present study evaluated the in vitro antimicrobial potential of the aqueous extract (AE) of Olea europaea against the main an etiological agent of dental caries Streptococcus mutans. The antimicrobial activity of O. europaea was evaluated by the disc diffusion method and to determine the susceptibility of the microorganism to the AE, the minimum inhibitory concentration (MIC) method was used. The mean zone of inhibition was observed to be 7.5 ± 1.74 mm at a concentration of 250 µg/mL. At all concentrations tested, S. mutans was significantly ($p < 0.05$) sensitive to O. europaea EC. O. europaea showed a growth inhibition of 67.4 ± 4.6 % at concentrations of 250 µg/mL with an IC50 value = 96.97 µg/mL. Our results indicate that the aqueous extract of *O. europaea* possesses antimicrobial activity against *S. mutans*.

**Keywords:** antimicrobial agents; plant extracts; natural products, caries.

## 1. Introduction

Oral diseases can cause pain, discomfort and dysfunction, negatively affecting quality of life (Vahabi et al., 2025). There are more than 500 bacterial species in human saliva and in white and hard tissues, some of them related to disease development (Yang & Kang, 2020). Dental caries is the most prevalent chronic bacterial disease in people worldwide (Golestannejad et al., 2020). The development of dental caries is related to the formation of cariogenic biofilms of oral pathogens on tooth surfaces (Zhang et al., 2022). *Streptococcus mutans* is the main etiological agent of dental caries and is naturally present in the oral cavity organized in oral microbial complexes. This Gram-positive bacterium synthesizes glucans to adhere to and interact with other bacterial species, resulting in the formation of a complex dental biofilm that promotes the development of caries (Zhang et al., 2022). S. mutans uses several virulence factors to promote the formation of cariogenic biofilms and the development of dental caries. Glycosyltransferases (Gtfs) are important virulence factors of *S. mutans*, which use sugars to synthesize glucans, thereby promoting bacterial cell attachment and biofilm formation (Yang & Kang, 2020). Several methods have been developed to control dental caries. Regular toothbrushing and flossing are the main methods for the prevention of dental caries. The adjunct of mechanical caries control procedures with antimicrobial agents for regular dental hygiene, such as chlorhexidine, sodium fluoride, metal nanoparticles and antimicrobial peptides, helps to reduce the population of cariogenic bacteria in biofilms (Zhang et al., 2022). However, the continued use of synthetic chemicals and antibiotics with conventional antimicrobial properties can lead to side effects such as cytotoxicity, mutagenicity and increased antibiotic resistance of most antimicrobial agents, creating the need for safe, cost-effective and efficient alternatives for the treatment of dental caries (Vahabi et al., 2025; Yang & Kang, 2020).

Medicinal plants are an important source of metabolites with diverse biological properties that are used as active ingredients for the treatment of diseases. They have been used for centuries and their importance for public health is

recognised by the World Health Organization. In recent years, secondary plant metabolites such as flavonoids, alkaloids, terpenoids, and saponins, among others, have been shown to be potent antimicrobial agents (Rodríguez-Garza et al., 2023). *Olea europaea* leaves and olive fruits have an ancient history of therapeutic and traditional practices as an essential part of Mediterranean culture due to polyphenols (Singer & Bourauel, 2021). Olive leaf extracts contain many of these bioactive polyphenols, which have various health benefits, such as antioxidant, anti-inflammatory, antitumour, hepatoprotective, neuroprotective, immunostimulant, antiviral and antimicrobial properties (Prevete et al., 2024). The aim of the present study was to evaluate the *in vitro* antimicrobial potential of the aqueous extract (AE) of *O. europaea* leaves against *S. mutans* (Figure 1).

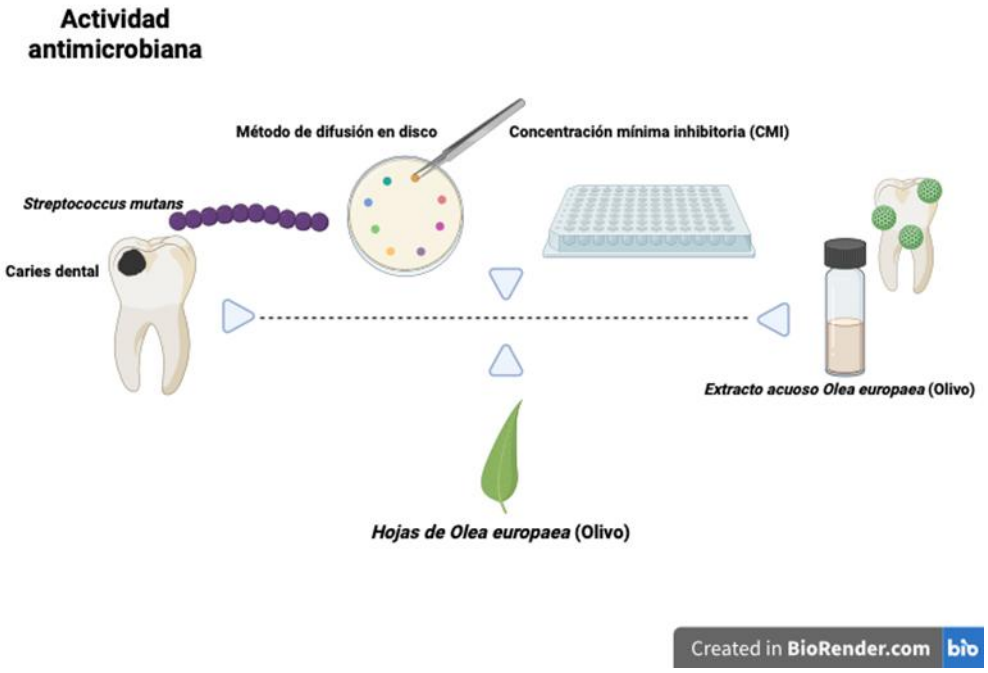


**Figure 1.** Antimicrobial activity of *Olea europaea* (olive).

## 2. Materials and Methods

*Plant material and extraction*

The plant was acquired in Pacalli® (pacalli.com.mx; Monterrey, Mexico) and a sample of the plant was deposited in the herbarium of the Faculty of Biological Sciences (FCB) of the Autonomous University of Nuevo León (UANL), Nuevo León, Mexico for correct taxonomic identification. Table 1 presents complete information on the plant species, family, botanical name, the specific part of the plant used for the preparation of the extract and the specimen voucher. Before extraction, the plant material was meticulously dried and ground in a mechanical mill into a fine powder. For extraction, 20 g of the powdered leaves of the plant were added to 500 mL of previously heated distilled water in a 1000 mL round flask to boiling point. Subsequently, a constant stirring (300 rpm) was applied until the infusion reached room temperature, after which it was filtered (Whatman™, Piscataway, USA) qualitative filter paper, grade 1) and distributed in freeze-drying bottles. Immediately afterwards, the extracts were frozen at -20 °C for 24 h and subjected to a freeze-drying process for 36 h (-50 °C / 0.2 mBar) in a laboratory freeze dryer (Freezone 2.5 Liter Benchtop Freeze Dryer, Labconco, Missouri, USA).

**Table 1.** Taxonomic identification of the medicinal plant used in this study.

| Plant Species* | Family | Common name | Part of the plant | Number voucher |
|---|---|---|---|---|
| *Olea europaea* | Oleaceae | Olive tree | Leaves | 026697 |

*The name and botanical family of the plant species were taxonomically validated using the website ThePlantList (https://www.theplantlist.org; accessed May 5, 2025).

*Bacterial culture*

The reference strain Streptococcus mutans Clarke UA130 (ATCC 700611™), which was acquired from the American Type Culture Collection (ATCC), the main an etiological agent of dental caries, was used in this research (Zhang et al., 2022). Culture and growth conditions of S. mutans UA130 were based on ATCC technical specifications, aerobic conditions at 37 °C for 24 h (Batubara et al., 2016). *S. mutans* was incubated for 6 h, at 37 °C, until the culture reached exponential growth (Thermo Scientific Lab-Line Incubator, USA).

*Antimicrobial activity*

The disc diffusion method was performed starting from a bacterial culture prepared under the same conditions as above until reaching the exponential growth phase (Wilkins & Thiel, 1973), 150 µL of inoculum was seeded on brain heart infusion agar plates (BHI, Becton Dickinson Bioxon®, Mexico) and a 6 mm filter paper disc (Whatman ™, Piscataway, USA) was placed with 20 µL of O. europaea EC (1000, 500, 250 and 125 µg/mL) (Alejandro Hernández-Marín et al. europaea (1000, 500, 250 and 125 µg/mL) (Alejandro Hernández-Marín et al., 2018a), on agar plates with S. mutans using a sterile needle. The positive control was 0.12% chlorhexidine gluconate (CHX, Ultradent Pro-ducts Inc., Consepsis™, South Jordan, UT, USA), commonly used in dentistry as a topical disinfectant in mouthwashes (Di Spirito et al., 2023). The culture plates were appropriately marked and incubated at 37 °C for 24h. Finally, the zone of inhibition around the disc was measured and expressed in mm (Hernández-Marín et al., 2018b). To determine the susceptibility of the microorganism to the aqueous extract, the minimum inhibitory concentration (MIC) method was used (Andrews, 2001). The concentration ranges were 1000, 500, 250 and 125 µg/mL.CHX was used as a positive control and distilled water as a negative control.S. mutans was pre-cultured as described above. A concentration of $10 \times 10^8$ CFU/mL of bacteria was inoculated into 96-well flat-bottomed microplates (COSTAR, Corning Inc., NY) containing TS medium and the extract. The extract was diluted appropriately to a final volume of 200 µL and then incubated at 37 °C for 24 h (Alejandro Hernández-Marín et al., 2018a) .After incubation, absorbance was measured at 570 nm (Thermo Scientific GENESYS 10 UV Scanning Spectrophotometer, WI, USA) (Ricardo et al., 2016)

*Statistical analysis*

All experiments were performed in triplicate ($n = 3$). Statistical analyses were performed using Graph Pad Prism 6 (GraphPad Software Inc., San Diego, CA). Mean values and standard deviation (SD) were calculated. Significant differences between *O. europaea* and CHX (0.12 %) were assessed using the one-way Anova test ($p \leq 0.05$) and a *post-hoc* Tukey test was applied to determine the difference between treatments.

## 3. Results

*Inhibition of the growth of Streptococcus mutans by the aqueous extract of Olea europaea*

The aqueous extract of *O. europaea* against *S. mutans* determined by the disc diffusion method showed that the mean zone of inhibition was 7.5 ± 1.74 mm at a concentration of 250 µg/mL. The positive control, CHX, showed inhibition zones of 9.0 ± 0.51 mm. In all concentrations tested, *S. mutans* was significantly ($p < 0.05$) more sensitive to the EO of *O. europaea*. *O. europaea* showed growth inhibition of 67.4 ± 4.6 % at concentrations of 250 µg/mL with a CI value of $_{50}$ = 96.97 µg/mL.

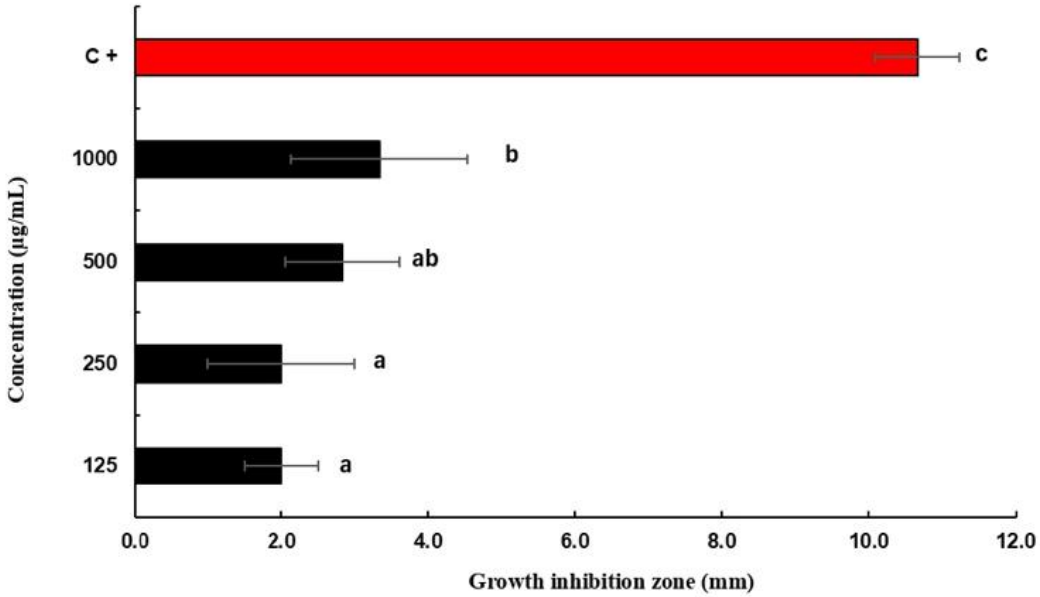


**Figure 2.** The data resulting from the inhibition halos of *S. mutans* in mm caused by the aqueous extract of *Olea europaea* (olive) valued at different concentrations (µg/mL) compared to the positive control (C+, CHX 0.12%) are shown. The data is shown as the average ± DS from three independent experiments. Different letters represent a significant high difference between the treatments determined by the 1-way Anova statistical test plus the *Tukey post-hoc* test.

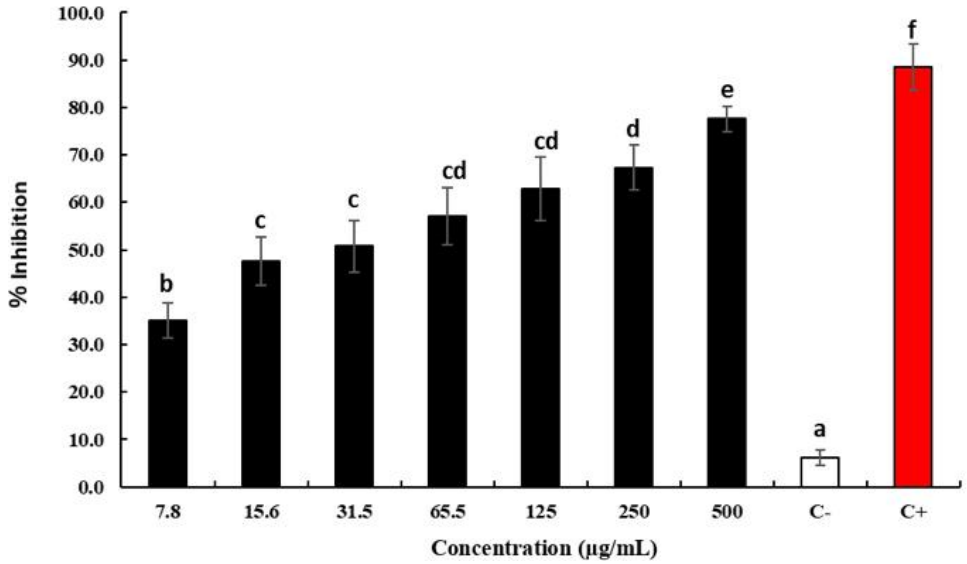


**Figure 3.** The data resulting from the percentage (%) of bacterial inhibition (*S. mutans*) caused by the extract of *O. europaea* evaluated at different concentrations in µg/mL and the negative and positive control are shown. The data is shown as the average ± DS from three independent experiments. Different letters represent a significant high difference between the treatments determined by the 1-way Anova statistical test plus the *Tukey post-hoc* test.

## 4. Discussion

In the present study we demonstrated that the EA of O. europaea has antibacterial activity against *S. mutans*, at a concentration of 250 µg/mL, the $IC_{50}$ values of the calculated EA was 96.97 µg/mL and the $IC_{90}$ was 325.54. Golestannejad et al. (2020) demonstrated the antibacterial activity of ethanolic, methanolic and hydroalcoholic extracts of *O. europaea*

on *S. mutans*, where the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were observed to be 12%-25%, 50%-75%, and 12%-25%, respectively; however, it is very likely that the ethanolic, methanolic and hydroalcoholic extracts of the same plant differ in composition (Golestannejad et al., 2020). On the other hand, in the study by Vahabi et al. reported that the hydroalcoholic extract of *O. europaea* exhibits antibacterial activity on *Aggregatibacter actinomycetemcomitans* with an MIC of 0.78% v/v (Vahabi et al., 2025). It has been reported that the methanolic extract of *O. europaea* has shown antibacterial activity against a wide range of oral pathogens, and the results show similarities with our study. Elnahas et al. reported that the ethanolic extract of *O. europaea* has activity against methicillin-resistant *Staphylococcus aureus* (MRSA), with an MIC of 15.6 mg/mL (Elnahas et al., 2021).

## 5. Conclusions

Our findings underscore the antimicrobial property of *the Olea europaea* EA. Further research, such as biodirected isolation of bioactive components, is warranted to assess their biological activity more comprehensively. In addition, to elucidate the molecular mechanisms related to its actions, and its potential application in the treatment of dental caries.

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## 6. Conflicts of interest

The authors declare that they have no conflict of interest.

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