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Antibacterial activity of *Baccharis dracunculifolia* in planktonic cultures and biofilms of *Streptococcus mutans*

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Summary *Streptococcus mutans* is an important cariogenic microorganism, and alternative methods for its elimination are required. Different concentrations of *Baccharis dracunculifolia* essential oil (EO) were tested to determine its minimal inhibitory concentration (MIC) in planktonic cultures, and this concentration was used in *S. mutans* biofilms. Additionally, we assessed the effect of a 0.12% chlorhexidine (CHX) and saline solution in *S. mutans* biofilms. The biofilms were grown in discs of composite resin for 48 h and exposed to *B. dracunculifolia*, CHX or saline solution for 5 min. The viability of the biofilms was determined by counting the colony-forming units per milliliter (CFU/ml) in agar, which was statistically significant ($P < 0.05$). The MIC of the *B. dracunculifolia* EO to planktonic growth of *S. mutans* was 6%. In biofilms of *S. mutans* clinical isolates, *B. dracunculifolia* EO (6%) and CHX resulted in reductions of 53.3—91.1% and 79.1—96.6%, respectively. For the

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biofilm formed by the *S. mutans* reference strain, the reductions achieved with *B. dracunculifolia* EO and CHX were, respectively, 39.3% and 88.1%. It was concluded that *B. dracunculifolia* EO showed antibacterial activity and was able to control this oral microorganism, which otherwise causes dental caries.

Introduction

A biofilm is a naturally occurring accumulation of microorganisms of either a single or multiple species embedded in an extracellular polymeric matrix and adherent to a biologic or non-biologic surface. Bacteria in a biofilm differ profoundly from their planktonic counterparts by growing in an enclosed, nutrient-sufficient ecosystem as a community of sessile organisms that exhibit an altered phenotype with respect to the growth rate, gene transcription [1], and resistance to antimicrobial agents [2]. The resistance of biofilms to antibiotics is increased compared with planktonic cells. In fact, when cells exist in a biofilm, they can become 10—1000 times more resistant to the effects of antimicrobial agents [3—5].

The oral cavity is heavily colonized by a complex, relatively specific and highly interrelated range of microorganisms, which are organized in biofilms. There is substantial evidence linking oral disease and respiratory tract infections with the pathogens in oral biofilms [6].

Approximately 20% of oral bacteria are streptococci [7]. The oral streptococci pioneer early dental plaque formation and have a specific temporal and spatial distribution that is crucial for the development of oral biofilms. *Streptococcus mutans* is considered to be the most cariogenic of all oral streptococci [8], and the main factors associated with its cariogenicity include adhesion, acidogenicity and acid tolerance [9,10]. These bacteria produce glucosyltransferases and synthesize glucans from sucrose, which mediate the adherence of *S. mutans* and other oral bacterial microbiota on tooth surfaces, contributing to the formation of dental biofilm. Selection for a cariogenic microbiota in dental biofilm increases the magnitude of the decrease in pH following the fermentation of available carbohydrates as well as increases the probability of enamel demineralization, which leads to the formation of dental caries [9].

Biofilms must be removed mechanically, but antibacterial mouth rinses are effective at decreasing tooth surface biofilms. In general, mouth rinses contain fluorides, alcohols, and detergents or antibacterial substances [11]. Ideal antibacterial substances must be effective against many microorganisms, act rapidly, maintain activity at low concentrations, lack side effects, and not cause discomfort [12]. Frequently used antibacterial chemicals include povidone iodine products, chlorhexidine, and cetylpyridinium chloride [13]. However, natural products have significantly contributed to the discovery of chemical structures and the creation of new medicaments that can be used as innovative therapeutic agents against this prevalent disease [14,15]

Baccharis dracunculifolia DC (Asteraceae) is a native plant from Brazil, which is commonly known as ''alecrim-do-campo''' and ''vassoura''. *B. dracunculifolia* is the most important botanical source of South-eastern Brazilian propolis, known as green propolis because of its color [16]. Teas, decoctions and tinctures prepared from the flowering plant are widely used in alternative medicine to treat inflammation, hepatic disorders and stomach ulcers [17]. A variety of chemical compounds and pharmacological activities have been attributed to this plant, including antiulcerative [18], antibacterial [19] and antifungal [20] properties.

The aim of this study was to determine the minimal inhibitory concentration (MIC) of *B. dracunculifolia* essential oil (EO) against planktonic cultures of *S. mutans* and its antibacterial activity in biofilms formed in the discs of composite resin.

Materials and methods

Microorganisms and standard suspensions

Seven *S. mutans* strains that had been previously isolated and identified from the oral cavities