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In vitro Evaluation of the Antimicrobial Potential of *Salvia officinalis* L. against Oral Pathogens

Avaliação *in vitro* do Potencial Antimicrobiano da *Salvia officinalis* L. Frente a Patógenos Orais

Carolina Medeiros de Almeida*[a]; Eveline Angélica Lira de Souza Sales Rocha[a]; Érika Ponchet Alves[a]; Rennaly de Freitas Lima[a]; Yuri Wanderley Cavalcanti[b]; Raquel Christina Barbosa Gomes[a]; Jozinete Vieira Pereira[a]; Edja Maria Melo de Brito Costa[a]

[a]State University of Paraíba, Department of Dentistry, Graduate Program in Dentistry. PB, Brazil.
[b]Federal University of Paraíba, Department of Clinical and Social Dentistry. PB, Brazil.
*E-mail: ccarol_21@hotmail.com
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Abstract

The emergence of multidrug-resistant strains to antibiotics has motivated the search for new substances with antimicrobial activity, especially those derived from medicinal plants. *Salvia officinalis* L. is a medicinal plant that arouses scientific interest due to being associated with multiple therapeutic effects. The purpose of this study was to evaluate the in vitro antimicrobial potential of *S. officinalis* L. against pathogens in the oral cavity. The antimicrobial potential of the ethanol extract of leaf of *S. officinalis* L was evaluated by broth microdilution, with determination of minimum inhibitory concentration (MIC) and Minimum bactericidal/Fungicide concentration (MBC / MFC), against the species *Streptococcus mutans, Streptococcus mitis, Streptococcus oralis, Streptococcus salivarius, Streptococcus sanguis, Candida albicans, Candida glabrata, Candida guillermond, Candida krusei and Candida tropicalis*. The extract showed moderate antifungal potential before *Candida species* (MIC = 1 mg/mL). And for the species of *Streptococcus*, the antimicrobial activity was from moderate to strong whose MIC ranged from 0.25 to 1 mg/mL. In this study, the extract from the leaves of *S. officinalis* L. presented oral cavity antimicrobial activity against pathogens. These results point to *S. officinalis* as a possible source of active ingredients in the development of formulations with antimicrobial activity of dental use.

Keywords: Plants, Medicinal. *Salvia officinalis* L. Products with Antimicrobial action.

Resumo

O surgimento de cepas multirresistentes a antibióticos tem motivado a busca de novas substâncias com atividade antimicrobiana, especialmente aquelas oriundas de plantas medicinais. A Salvia officinalis L. é uma planta medicinal que desperta interesse científico por estar associada a múltiplos efeitos terapêuticos. O objetivo do presente estudo foi avaliar o potencial antimicrobiano in vitro da S. officinalis L. frente a patógenos da cavidade bucal. O potencial antimicrobiano do extrato etanólico da folha da S. officinalis L foi avaliado por meio da microdiluição em caldo, com determinação da Concentração Inibitória Mínima (CIM) e Concentração Bactericida/Fungicida Mínima (CBM/CFM), frente as espécies Streptococcus mutans, Streptococcus mitis, Streptococcus oralis, Streptococcus salivarius, Streptococcus sanguis, Candida albicans, Candida glabrata, Candida guillermond, Candida krusei e Candida tropicalis. O extrato apresentou moderado potencial antifúngico frente às espécies de Candida (CIM = 1 mg/mL). Para as espécies de Streptococcus, o potencial antimicrobiano foi considerado forte a moderado, com valores de CIM variando entre 0,25 a 1 mg/mL. Neste estudo, o extrato da folha de S. officinalis L. apresentou potencial antimicrobiano contra patógenos da cavidade bucal. Esses resultados apontam a S. officinalis como uma possível fonte de princípios ativos no desenvolvimento de formulações com atividade antimicrobiana de uso odontológico.

Palavras-chave: Plantas Medicinais. Salvia officinalis L. Produtos com Ação Antimicrobiana.

1 Introduction

The development of more frequent oral pathologies, such as dental caries, periodontal disease and oral candidiasis, is directly linked to the formation of oral biofilm, constituted especially by bacteria and yeasts[1]. Therefore, the physical and chemical control of biofilm, characterized by brushing and use of substances with antimicrobial potential, is the main method of prevention of associated diseases. An antibiofilm substance must reduce its formation, to be harmless to oral tissues, not to stain the teeth, not to change the taste and not to favor the emergence of microbial resistance[2]. However, there is not currently any product available in the market that fulfills all these characteristics. Thus, it is identified the need for research and development of new products, which can combat, inhibit or reduce the oral pathogenic microbiota, to ensure its safe indication for the general population[3].

Moreover, the onset of microbial resistance on the part of some species that cause these oral diseases. Condition that has increased in recent years, establishing the need for development of new therapeutic methods and antimicrobial strategies[4]. In this perspective, the interest of the scientific community by medicinal plants are rich sources of active principles with biological properties, whose potential antimicrobial agent has been investigated with the objective of treating or reduce oral infections, to ensure its safe indication for the general population[3,5-8].

Among the available biodiversity, what stands out is the *Salvia officinalis* L., one of the most used plant species in traditional medicine, in function of its biological properties, as

an antioxidant[9,10]antimicrobial[10,11], anti-inflammatory[12,13], and antitumor[9,14], resulting from the action of various chemical compounds present in the plant, including diterpenes, triterpenes, flavonoids[15] and phenolic acids[16].

S. officinalis belongs to the family of *Laminaceas*, which originates in the Eastern Mediterranean region and cultivated in several countries of temperate climate and with plenty of light. In Brazil, it is popularly known as salvia, *salva-das-boticas*, yerba santa or salvamansa[11] and although not originally Brazilian, has good adaptation in the country mainly in the southern region[17].

Despite its use being widespread and its chemical characterization and antimicrobial activity documented in some studies[16-21], there is still the need of scientific studies that demonstrate the use of this plant on oral diseases[22]. In this sense, considering the need for new studies about the antimicrobial effect of *S. officinalis*, the aim of this work was to evaluate *in vitro* the antimicrobial potential of this plant outside the oral pathogens.

2 Material and Methods

2.1 Preparation of plant material

The ethanolic extract of dried and crushed leaves of *S. officinalis*. was obtained by the process of maceration for 48 hours at room temperature, using the proportion of 10 g of plant to 75 mL of ethyl alcohol solvent (96%). The resulting mixture was filtered, and the waste immersed two more times, in 75 mL of alcohol 96%. The final three liquid phases were concentrated in a rotary evaporator under reduced pressure at a temperature of 50ºC. For the microbial susceptibility tests, the extract was diluted in alcohol at 40%, at a concentration of 8 mg/mL. The final product was kept in the refrigerator and protected from light.

2.2 Microorganisms and evaluation of antimicrobial activity

The micro-organisms included in this study were: *Streptococcus mutans* ATCC 25175, *Streptococcus mitis ATCC 903, Streptococcus oralis* ATCC 10557*, Streptococcus salivarius* ATCC 7073*, Streptococcus sanguis* ATCC 10557*, Candida albicans* ATCC 18804, *Candida glabrata* ATCC 2001*, Candida guillermond* ATCC 6260*, Candida krusei* ATCC 34135 *and Candida tropicalis* ATCC 13803.

The antimicrobial activity of ethanolic extract of *S. officinalis* L. was verified by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations fungicide (CFM and CBM), in accordance with the standards M27-A3 and M7-A8 Clinical and Laboratory Standards Institute (CLSI)[23,24]. The test was performed in 96-well microplates, containing 100 µL/well of culture medium (Brain Heart Infusion - Himedia® - for bacteria and Sabouraud Dextrose Agar - Himedia® - for the yeasts). The substances previously diluted in alcohol (40% v/v; 8 mg/mL), were transferred to the first well, being subsequently performed serial dilutions in order to obtain concentrations between 2 mg/mL and 0.01562 mg/mL. Positive controls were chlorhexidine 0.12% (Sigma-Aldrich®) and nystatin (Sigma-Aldrich®) and the negative control alcohol 40%. The bacterial ($1.0x10^6$ CFU/mL) and fungal inoculants($5.0x10^3$ CFU/mL) were added to the wells and the plates incubated at 37°C for 24 hours. CIM was defined as the lowest concentration of the extract that inhibits visible microbial growth, confirmed by resazurin 0.01% (Sigma-Aldrich®). To determine CBM/CFM, an aliquot of 50 µL of each well, with concentrations equal to or greater than CIM, were sub cultivated in BHI broth - Himedia® (for bacteria) or Sabouraud Dextrose Agar - Himedia® (for yeast) and incubated at 37°C for 24 hours. CBM/CFM were defined as the lowest concentration that inhibited visible growth in solid medium.

3 Results and Discussion

The results of CIM, CBM and CFM of ethanolic extract of *S. officinalis* leaf are shown in Tables 1 and 2, respectively. The extract presented antimicrobial activity on all species of bacteria of the genus *Streptococcus* tested, mainly on the species *S. mutans*, *S mitis* and *S sanguis*, whose CIM was 0.25 mg/mL. The yeasts of the genus *Candida* also proved to be sensitive to the extract, presenting MIC of 1 mg/mL for all the tested species. For the nystatin and chlorhexidine, the CIMs were 0.0156 mg/mL.

Table 1 - Minimal inhibitory concentration and minimum bactericidal concentrations of chlorhexidine (0.12%) and of the ethanolic extract of *S. officinalis* against species of *Streptococcus*.

Bacteria	*Salvia officinalis* L.		Chlorhexidine (0.12%)
	CIM (mg/mL)	CBM (mg/mL)	CIM (mg/mL)
***Streptococcus mutans* ATCC 25175**	0.25	1.0	0.0156
***Streptococcus mitis* ATCC 903**	0.25	1.0	0,0156
***Streptococcus sanguis* ATCC 10557**	0.25	> 2	0.0156
***Streptococcus salivarius* ATCC 7073**	0.5	1.0	0.0156
***Streptococcus oralis* ATCC 10557**	1.0	2.0	0.0156

Source: Research data.

Table 2 - Minimal inhibitory concentration and minimum fungal concentration of nystatin and ethanolic extract of *S. officinalis* against species of *Candida*.

Yeasts	*Salvia officinalis* L.		Nystatin
	CIM (mg/mL)	CFM (mg/mL)	CIM (mg/mL)
***Candida albicans* ATCC 18804**	1.0	2.0	0.0156
***Candida glabrata* ATCC 2001**	1.0	1.0	0.0156
***Candida guillermond* ATCC 6260**	1.0	2.0	0.0156
***Candida krusei* ATCC 34135**	1.0	2.0	0.0156
***Candida tropicalis* ATCC 13803**	1.0	1.0	0.0156

Source: Research data.

For the evaluation of the antimicrobial potential of the extract of *S. officinalis* species were used of bacteria and yeasts, which are usually associated with the development of caries and oral candidosis, the antimicrobial potential of *S. officinalis* was evaluated before some species of bacteria and yeasts, with positive results mainly on *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* , *Streptococcus mutans* and *Candida albicans*[11,18-20,25-27]. Thus, the results of this study corroborate the literature to demonstrate the antimicrobial effect on wide variety of microorganisms.

It was observed that the extract of *S. officinalis* has antimicrobial activity against species of *Streptococcus*, considered strong (*S. mutans*, *S. mitis*, *S. sanguis*: CIM=0.25 mg/mL) to moderate (*S. salivarius:* CIM=0.5mg/mL) *S. oralis:* CIM=1 mg/mL), according to the classification established by Aligiannis et al[28]. The extract of *S. officinalis* showed bacteriostatic profile, with inhibition of the growth of the microorganism without causing its death, which can be positive, if considered the imbalance of the oral microbiota produced by antibacterial substances. Similar results were found by Moreira et al[26] that through the technique of broth microdilution, verified that the crude extract of the leaf of *S. officinalis* and its diterpene-manool fraction have strong antibacterial activity, mainly on the *S. mutans*, with CIM between 0.00624 and 0.03136 mg/mL.

The results of the antimicrobial activity of *S. officinalis* on the species of *Streptococcus* are promising, especially when one considers the biogenesis of dental biofilm. It is known that this biofilm is composed of a variety of microbiological communities contained in a polymer matrix of salivary and bacterial origin, formed particularly by *oral Streptococci*[29]. *S. mutans* is the most related species to the development of dental caries, depending on its ability to adhere to the tooth surface and produce acids from fermentation of carbohydrates, propitiating the demineralization of dental enamel[30.31]. Furthermore, despite *S. mutans* being the key bacterium in the dental caries process, dental biofilm is initially colonized by other species, such as *Streptococcus mitis, Streptococcus sanguis, Streptococcus oralis, Streptococcus salivarius, Streptococcus sobrinus* and *Lactobacillus casei,* being crucial in this pathogenicity process, causing damage to the gingival and dental tissues[32].

Due to being an organized, proliferative, enzymatically active association and with a capacity of adhesion to the teeth surface, which may generate pathological changes in the oral cavity, the biofilm must be disorganized as quickly and efficiently as possible, and it is possible to associate the measures of mechanical removal, the methods of chemical control of dental biofilm[32]. Among the chemical agents available in the market for the prevention of diseases related to the dental biofilm, chlorhexidine stands out, considered an antiseptic of wide spectrum. It acts on fungi and gram-positive and gram-negative bacteria[33] and has good efficacy in removal of cariogenic biofilm[34]. On the other hand, this substance presents limitations such as changes of taste, stains on the teeth and an imbalance in the oral microbiota[35] and although has great effectiveness on microorganisms, some already present resistance[36].

It was also observed, in this study, a moderate antifungal potential[28] before species of the genus *Candida* (CIM=1 mg/mL). *S. officinalis* showed fungistatic profile, with values of CFM, between 1-2 mg/mL, inhibiting the visible growth of yeasts. The analyzed *Candida* species are involved in the etiology of oral candidosis, an opportunistic disease that most commonly affects immunocompromised individuals, being *C. albicans* the most associated species to infection[37.38]. The drug of choice for the treatment of candidiasis is the nystatin, which despite of being widely used, there are already reports of resistance of some species of *Candida*[39.40]. The record of infections caused by *Candida* has displayed a growing resistance to antifungals, leading to failures in the treatment and recurrent infections[6].

Using the disk diffusion method, Garcia et al.[11] did not identify the antimicrobial potential of the extract of *S. officinalis* against *C. albicans* and *C. tropicalis*, differing from the findings of this study, which showed antifungal activity on all strains of the tested *Candida*. This difference in results can be attributed to various factors, related to the plant, the micro-organism, the method used, among others. An important factor to be considered when research is carried out involving medicinal plants are the environmental conditions, such as seasonality, climate, soil type and temperature of the air. The production of secondary metabolites by the plant occurs in function of the plant's interaction with the environment in response to chemical and biological factors[41].

The literature presents also records of the antifungal activity of the essential oil of *S. officinalis*. Badiee et al.[15] using the method of broth microdilution, observed the activity on standard strains of *C. albicans, C. parapsilosis and C. krusei* and recent clinical isolates of *C. albicans* and *C. glabrata*, with CIM equal to 0.0156, 0.0039, 0.0313, 0.0313 and 0.0019 mg/ml, respectively. With these results, the authors pointed out *S. officinalis* as natural alternative for the treatment of candidiasis. Sookto et al[27] also assessed the activity of the essential oil of *S. officinalis* on *C. albicans* and observed the formation of inhibition halos of 19.5 to 40.5 mm and MIC of 2.780 g/L, confirming the antifungal activity against yeast. It is highlighted that the results found in in vitro test may not correspond to the actual behavior of products tested *in vivo,* since they are not exposed to the same conditions of the oral cavity[42].

4 Conclusion

The results of this study show that the ethanolic extract of *S. officinalis* L. presents a strong antibacterial activity on bacteria of the genus *Streptococcus*, closely related to the etiology of dental caries, in addition to moderate antifungal activity before *Candida* species . These results reinforce the

importance of the therapeutic indications of medicinal plants and suggest the implementation of other pre-clinical and clinical methods, in order to define the mechanism of action and its possible effectiveness in the control of oral biofilm, prior to their use in dental clinic.

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