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Antibacterial and anti-adherent effect of *Mimosa tenuiflora* and *Myrciaria cauliflora* on dental biofilm bacteria

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The use of plants in the prevention and treatment of oral infectious and as an antibiofilm agent continues to be valued in many parts of the world. The aim of study is to evaluate *in vitro* antimicrobial action of the plant extracts of barks *Mimosa tenuiflora* (Willd.), Poir. (jurema preta) and leaf and stem of *Myrciaria cauliflora* Berg. (jabuticabeira) against dental biofilm bactéria. The oral bacteria were used to determine the minimum inhibitory concentration (MIC) and minimum inhibitory concentration of adherence (MICA): *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus salivarius* and *Lactobacillus casei*. Each assay was carried out in duplicate and the positive control (0.12% chlorhexidine digluconate) was subjected to the same procedure. Results were analyzed by Student t test or Mann-Whitney test, with the level of significance set at 5%. The extract of *M. tenuiflora* showed inhibition halos ranging from 10 to 25 mm in diameter, presenting an average performance superior to chlorhexidine digluconate, being statistically significant only at the concentration of 1:128 (3.9 mg/mL). The leaf and stem extracts of *M. cauliflora* were found to have inhibition halos varying from 10 to 18 mm in diameter and presented a significantly lower average performance in relation to chlorhexidine digluconate in crude extract (500 mg/mL) concentration; 1:2 (250 mg/mL) and 1:4 (125 mg/mL) and crude extract (500 mg/mL), 1:2 (250 mg/mL), 1:4 (125 mg/mL) and 1:8 (62 mg/mL), respectively. All extracts studied were effective in the inhibition of adherence, especially the stem extract of *M. cauliflora* (1:64/ 7.81 mg/mL). Conclusively, the extracts of *M. tenuiflora* and *M. cauliflora* produced a significant bactericidal activity and *in vitro* anti-adherent effect on the bacteria forming the dental biofilm, which suggests the use of these substances as an alternative and economically viable means for the control of infections in dentistry.

Key words: Microbiology, phytotherapy, *Mimosa tenuiflora* (Willd.) Poir., *Myrciaria cauliflora* Berg.

INTRODUCTION

In oral environment, microorganisms are organized as biofilm. The formation of biofilm follows a regular pattern, involving the initial association of pathogens with salivary pellicle in enamel, followed by interbacterial participation in a process known as secondary colonization (Cavalcanti et al., 2014; Lobo et al., 2015).

Oral infectious diseases can be prevented by disruption of the biofilm using mechanical or chemical methods. Various antimicrobial agents are used in the chemical control of biofilms, such as cationic agents, chlorhexidine and cetylpyridinium chloride, which immediately bind to the bacterial surface, negatively charged, while the triclosan and phenolic compounds are non-ionic agents (Ocheng et al., 2014; Vargas et al., 2015; Costa et al., 2017). Chlorhexidine is the most common anti-biofilm agent used in dentistry (Araújo, 2015; Vieira et al., 2014). It acts in the general disruption of the cell membrane and in the specific inhibition of membrane enzymes, which inhibits glucose uptake by *Streptococcus mutans* and uses lactic acid for its metabolism, as well as reducing the proteolytic activity of *Porphyromonas gingivalis*. Its action is unquestionable, but chlorhexidine has negative effects such as gustatory interference, mottling of the tooth surface and restoration, which may even cause microbial resistance (Hajifattahi et al., 2016; Salleh et al., 2011). Increasing antimicrobial resistance, adverse effects and evolution of new species of disease-causing bacteria, have shown a need for finding new and safe antibacterial agents. Medicinal plants is an attractive source for new discoveries of antibacterial agents, given their molecular diversity (Majali et al., 2015; Hajifattahi et al., 2016; Al-Ayed et al., 2016; Alagl et al., 2017). In Brazil, there is approximately 25% of the 250,000 medicinal species cataloged by UNESCO, and a little more than eight thousand species are in the Brazilian semiarid region, facilitating the access and use of the healing potential of these plants in the treatment of diseases. These include the area of dentistry, specifically, diseases dependent on biofilm, such as caries and gingivitis. In recent years, some studies have been developed in Brazil in order to assess the popular use of vegetation in dentistry, making it possible to identify species with potential antimicrobial activity (Majali et al., 2015; Vieira et al., 2014; Sette - de -Souza et al., 2014). Several studies with plants of Caatinga in Northeastern Brazil, including Jurema preta (*M. tenuiflora*) and Jabuticabeira (*M. cauliflora*) show the presence of some secondary metabolites, such as tannins and phenolic compounds. Such substances have significant antimicrobial activity and may act in activating enzymes which are responsible for important pharmacological effects such as anti-inflammatory, antimicrobial, antioxidant, and others (Bezerra et al., 2011; Bona et al., 2014; Azevedo et al., 2015). According to de Oliveira at al. (2012), phenolic compounds are associated with various medicinal effects. The objective of this study was to evaluate the antibacterial activity and non-stick effect of plant extracts of barks *M. tenuiflora* (Willd.) Poir. (jurema preta) and leaf and stem of *M. cauliflora* Berg. (jabuticabeira) in the control of microorganisms of biofilm related to tooth decay, the most prevalent chronic disease.

MATERIALS AND METHODS

Preparation of the *M. tenuiflora* (Willd.) Poir and *M. cauliflora* Berg. Extract

Barks of *M. tenuiflora* (Willd.) Poir and leaf and stem of *M. cauliflora* Berg. were collected in the town of Teixeira, Paraíba.

The crude extract was prepared at the Laboratory of Chemical and Biological Sciences, Federal University of Campina Grande (UFCG), Center for Health and Rural Technology (CSTR). A voucher specimen of the plant was deposited at the Dárdano de Andrade Lima Herbarium, Regional University of Cariri (URCA), Crato, Ceará (Registration No. 4016).

After collection, the *M. tenuiflora* (willd.) poir. (barks) and *M. cauliflora* Berg. (leaf and stem) samples were desiccated in an oven in circulating air at an average temperature of 45°C and then ground to powder in a mechanical grinder. The dry and ground material was macerated with 2 L of 95% ethanol for 72 h. The resulting crude *M. tenuiflora* (willd.) poir. and *M. cauliflora* Berg. extract was concentrated in a rotary evaporator under reduced pressure at a temperature that did not exceed 40°C.

Determination of minimum inhibitory concentration

Antibacterial activity was evaluated *in vitro* according to the method of Bauer et al. (1966) for the determination of minimum inhibitory concentration (MIC). The following bacterial strains were cultured in brain heart infusion broth (BHI; Difco, Detroit, MI, USA) at 37°C for 18 to 20 h under microaerophilic conditions: *Streptococcus mitis* (ATCC 903), *S. mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 15300), *Streptococcus oralis* (ATCC 10557), *Streptococcus salivarius* (ATCC 7073), and *Lactobacillus casei* (ATCC 9595) (bacteria of the resident microbiota and dental caries).

Saline inoculated with each bacterial growth was spread across petri dishes containing Mueller-Hinton agar (Difco) and five standard holes measuring approximately 6 mm in diameter were punched in each plate. Next, 50 µL of the test substance (crude extract diluted in distilled water up to 1:512) was added to the holes and the plates were incubated at 37°C for 24 h under microaerophilic conditions.

Each assay was carried out in duplicate for each strain. The same procedure was used for positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA).

Inhibition halos (in mm) were measured with caliper (Digimess®, São Paulo, Brazil) and MIC was defined as the lowest concentration of the extract that was able to inhibit bacterial growth. The results were transferred to database and Kolmogorov-Smirnov and Levene tests were used. After that, data were analyzed by the Student t-test or Mann-Whitney test, with the level of significance set at 5%.

Determination of minimum inhibitory concentration of adherence

The minimum inhibitory concentration of adherence (MICA) of *M.*

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Table 1. Student t-test or Mann-Whitney U test (MIC *M. tenuiflora* extract vs.chlorhexidine digluconate).

Concentrations/dilutions of extract/chlorhexidine (mg/mL)	MIC – Mean		Statistic t	Significance
	Extract	Chlorhexidine		*p*-value
CE (500) vs. CD	21.5	21.0	0.361	0.725
1:2 (250)	19.5	19.3	0.159	0.878
1:4 (125)	19.2	17.8	1.105	0.295
1:8 (62.5)	18.3	16.5	1.467	0.173
1:16 (31.25)	16.8	14.3	1.612	0.153
1:32 (15.65)	14.7	11.0	1.46	0.201
1: 64 (7.81)	13.5	6.3	2.469	0.055
1:128 (3.9)	12.0	5.0	3.09	0.025*

CE: crude extract CD: chlorhexidine digluconate * (*p*-value < 0,05) (significant results).

Table 2.. Student t-test or Mann-Whitney U test (MIC *M. cauliflora* extract vs.chlorhexidine digluconate).

Concentrations/dilutions of extract/chlorhexidine (mg/mL)	MIC – Mean		Statistic t	Significance
	Extract	Chlorhexidine		*p*-value
CE (500) vs. CD	16.3	21.0	-3.715	0.008*
1:2 (250)	13.7	19.3	-5.271	0.001*
1:4 (125)	9.2	17.8	-4.046	0.002*

CE: crude extract CD: chlorhexidine digluconate * (*p*-value < 0,05) (significant results).

tenuiflora (willd.) poir. (Stem) and *M. cauliflora* Berg. (Leaf and Stem) extract was determined in the presence of 5% sucrose as described by Gerbara et al. (1996) using concentrations, corresponding to the crude extract and diluted up to 1:512. *S. mitis* (ATCC 903), *S. mutans* (ATCC 25175), *S. sanguinis* (ATCC 15300), *S. oralis* (ATCC 10557), and *S. salivarius* (ATCC 7073) were sub-cultured in Mueller-Hinton broth (Difco) at 37°C (Table 3). Subsequent, 1.8 mL of the sub-culture was transferred to hemolysis tubes and 0.2 mL of the different dilutions of the extract was added.

Adhesion of bacteria to tube after shaking was analyzed visually after 24 h. Each assay was carried out in duplicate for each selected strain. The same procedure was used for positive control (0.12% chlorhexidine digluconate; Periogard®, Colgate-Palmolive Company).

The MICA is defined as the lowest concentration of the extract in medium with sucrose which prevented adhesion to the glass tube.

RESULTS

Determination of minimum inhibitory concentration

Hydroalcoholic extract of bark of M. tenuiflora

After data collection, the data were tabulated and analyzed statistically. Considering the extracts tested, the Student t-test was used at 5% significance level in the comparison between the hydroalcoholic extract of bark *M. tenuiflora* and 0.12% chlorhexidine gluconate (two independent groups), as shown in Table 1.

Hydroalcoholic extract of leaf M. cauliflora

At 5% significance level, the Student t-test was used to compare the hydroalcoholic extract of leaf *M. cauliflora* and 0.12% chlorhexidine gluconate as shown in Table 2.

Hydroalcoholic extract of stem M. cauliflora

At 5% significance level, the Student t-test was used to compare the hydroalcoholic extract of stem *M. cauliflora* and 0.12% chlorhexidine gluconate as shown in Table 3.

Determination of minimum inhibitory concentration of adherence

The test results of MICA extracts and 0.12% chlorhexidine gluconate are shown in Table 4.

DISCUSSION

Currently, there is an interest from the scientific community in medicinal plants with proven antimicrobial activity, especially with the increasing awareness of the side effects of traditional drugs. The undesirable effects of synthetic drugs and the difficulty in producing new drugs effective in microbial combat suggest that the use

Table 3.. Student t-test or Mann-Whitney U test (MIC *M. cauliflora* extract vs.chlorhexidine digluconate).

Concentrations/dilutions of extract/chlorhexidine (mg/mL)	MIC – Mean		Statistic t	Significance
	Extract	Chlorhexidine		(*p*-value)
CE (500) vs. CD	16.8	21.0	-3.201	0.009*
1:2 (250)	15.0	19.3	-4.24	0.005*
1:4 (125)	12.7	17.8	-4.706	0.004*
1:8 (62.5)	10.5	16.5	-5.555	0.002*

CE: crude extract CD: chlorhexidine digluconate * (*p*-value < 0,05) (significant results).

Table 4. Determination of minimum inhibitory concentration of adherence of the extracts and 0.12% chlorhexidine gluconate

Dental biofilm bacteria	*M. tenuiflora* extract (MICA)	*M. cauliflora* extract stem (CIMA)	*M. cauliflora* extract leaf (CIMA)	Chlorhexidine digluconate
Streptococcus mitis	1:8	1:4	1:8	1:128
Streptococcus mutans	1:4	1:4	1:8	1:256
Streptococcus sanguinis	1:8	1:8	1:4	1:64
Streptococcus oralis	1:16	1:64	1:32	1:32
Streptococcus salivarius	1:8	1:16	1:16	1:64
Lactobacillus casei	0	0	0	1:64

of natural plant extracts in fighting the colonizing microorganisms of biofilm should be investigated (Upreti et al., 2012; Al-Ayed et al., 2016).

An initial selection of the antimicrobial potential should be done with the natural plant. The most common way to perform this study is through extract dilutions and test them on disks or wells made in petri dishes. These methods have scientific merit and are recognized to be of great value for studies of infectious diseases (Moreira et al., 2012; de Oliveira et al., 2013). In the present study, we used microorganisms which form biofilm and are resilient to antimicrobials. Pathogens were tested *in vitro* to determine the antimicrobial activity and non-stick of hydroalcoholic extracts from the bark of *M. tenuiflora* (Willd.) Poir. and leaf and stem of *M. cauliflora* Berg.

The results of the Student t-test showed that the hydroalcoholic extract from the bark of *M. tenuiflora* (Willd.) Poir had an average performance higher than 0.12% chlorhexidine gluconate. The data obtained in this test showed a great potential for *M. tenuiflora* (Willd.) Poir on microorganisms of the biofilm which confirms the results obtained in previous trials (Bezerra et al., 2009).

All strains proved to be sensitive to leaf of *M. cauliflora* extract, but the results of Student t test showed that the 0.12% chlorhexidine digluconate had an average performance significantly higher than the hydroalcoholic extract of *M. cauliflora* Berg leaf. The stem extract from *M. cauliflora* Berg. formed inhibition zones ranging from 10 to 20 mm, which agrees with studies by Bonn et al., (2014), where extracts ranged from 6.5 to 12.5 mm in various microbial species. All samples also showed sensitivity to stem of *M. cauliflora* extract at a dilution of 1:8 (62.5 mg/mL), but for *S. mitis*, *S. oralis* and *S. Salivarius*,the inhibition zone was 11 mm, while for *S. mutans*, *S. sanguinis* and *L. Casei*, it was 10 mm.

The data demonstrates the effectiveness of all extracts of *M. cauliflora*, showing the potential of these substances as antimicrobial agents. The bacteriostatic and bactericidal activity in the *M. cauliflora* extract may be related to the presence of the compounds of its main constituent class, tannins, and substances with significant antimicrobial activity. The formation process of the biofilm starts basically with the adherence of microorganisms to the tooth surface, further highlighting the importance of the non-stick ability of antimicrobials to act in the early stages of biofilm formation (Cavalcanti et al., 2014).

The results of these studies demonstrated the greater effectiveness of 0.12% chlorhexidine digluconate on the inhibition of adhesion on all tested strains, compared to that presented by the extract of *M. tenuiflora*. The *M. cauliflora* leaf extract and chlorhexidine digluconate inhibit the adhesion of *S. oralis* at the same concentration of 1:32 (15.65 mg/mL), while *M. cauliflora* stem extract showed greater effectiveness on inhibition of *Streptococcus oralis* at 1:64 (7.81 mg/mL).

The collected data demonstrate the high potential of antimicrobial and non-stick activity of the plant extracts, mainly *M. tenuiflora* on all tested microorganisms, suggesting the possibility of using this agent in concentrations which meet the MIC and MICA in the oral environment. Inhibition of glucan synthesis and its

bacteriostatic action enables these extracts to take effect in controlling biofilm buildup previously established and therefore prevent tooth decay and gingivitis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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