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Original Article

Antimicrobial and Anti-Adherent in vitro Activity of Tannins Isolated from Anacardium occidentale Linn. (Cashew) on Dental Biolfilm Bacteria

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Abstract

Objective: To evaluate the antimicrobial and ant-adherent in vitro activity of tannins isolated from Anacardium occidentale Linn. (Cashew) on dental biofilm bacteria. **Material and Methods:** Streptococcus mutans ATCC 25175, Streptococcus mitis ATCC 903, Streptococcus sanguis ATCC 15300, Streptococcus oralis ATCC 10557, Streptococcus salivarius ATCC 7073 and Lactobacillus casei ATCC 9595 samples were used in this study. The tests were performed by the solid medium dilution method to determine the Minimum Inhibitory Concentration (MIC). The Minimum Inhibitory Concentration of Adherence (MICA) of bacteria to glass was determined in the presence of 5% sucrose. As a positive control, 0.12% chlorhexidine gluconate was used. The substances were tested at concentrations of 1:1 (pure solution) up to 1:512. Data were analyzed using descriptive statistics and the SPSS software, version 15.0. **Results:** Tannins isolated from Anacardium occidentale Linn. (cashew) formed inhibition halos ranging from 11 to 17 mm in diameter and were capable of inhibiting the growth of bacteria tested at concentrations of 1:4 (S mutans), 1:16 (S mitis), 1:8 (S sanguis), 1:4 (S oralis), 1:8 (S salivarius) and 1:2 (L casei). The tannin solution was effective in inhibiting the adherence of microorganisms to glass, and its effect on Streptococcus sanguis (1:512) and Lactobacillus casei (1:512) stood out, showing ant-adherent effect at all concentrations tested. **Conclusion:** Tannin isolates produced in vitro antimicrobial and ant-adherent activity on dental biofilm-forming bacteria and can be considered as an alternative treatment in infectious processes in clinical dentistry.

Keywords: Phytotherapy; Dental plaque; Preventive dentistry..

Introduction

Dental Poor control of dental biofilm is considered the most important factor for the prevention and treatment of oral diseases such as caries, gingivitis and periodontal disease, with a very strong relationship with poor oral hygiene [1]. Thus, the inability of much of adult population to carry out an effective oral hygiene has encouraged research with chemical agents that can assist in the control of dental biofilm [2,3].

Among the chemical agents used to assist in the control of dental biofilm, chlorhexidine gluconate stands out, which has been indicated due to its effectiveness in the chemical removal of cariogenic or periodontal biofilm. However, due to the adverse effects of this agent such as staining, taste change, microbiota imbalances, other agents, including those of natural origin, have been investigated [4]. Thus, studies using plant products have increased and there are promising results, especially for oral diseases related to dental biofilm accumulation [3,5].

Anacardium occidentale Linn., an important member of the Anacardiaceae family popularly known as "cashew", is a tropical tree native to northeastern and northern Brazil [6,7]. This medicinal plant has been popularly used for treating urogenital infections, diarrhea, and hypotension as antidiabetic agent, as well as for the treatment of gastrointestinal disorders such as mouth ulcers, tonsillitis, bronchitis, arthritis, toothache, and gingivitis [8,9].

The Anacardium occidentale bark is astringent [6] and in vitro studies using hydroalcoholic extract has shown that this part of the plant has antimicrobial activity against oral pathogens that form dental biofilm and may be therapeutically used in dentistry as an antibacterial agent [10,11].

Laboratory studies have identified that Anacardium occidentale stem bark presents large amounts of anacardic acids and tannins, suggesting its potential anti-oxidant activity [6]. Vegetable tannins are defined as water-soluble phenolic compounds (polyphenols) with the ability to precipitate alkaloids, gelatins and proteins. Many of the pharmacological actions of tannins seem to derive from their ability to form complexes with proteins and polysaccharides. Thus, they contribute to the healing of wounds and burns, forming a film of polyphenols associated to proteins or polysaccharides under which the natural healing process occurs. Similarly, the affinity of polyphenols for proteins plays an important role in the inactivation of enzymes, thus preventing the growth of some microorganisms [12].

The action of tannins on the membrane and cell organelles of microorganisms has been reported in a previous study [13]. According to the author, tannins may act by inhibiting extracellular microbial enzymes, interfering with the availability of substrates required for bacterial growth or directly acting on microbial metabolism through inhibition of oxidative phosphorylation, suggesting that these mechanisms are responsible for their bactericidal properties.

Based on the above, this study aims to evaluate the in vitro antimicrobial and ant-adherent activity of tannins isolated from cashew stem bark on dental biofilm-forming microorganisms, since studies have evaluated the activity of the crude extract, and not of isolated compounds, which are really responsible for microbial growth inhibition.

Material and Methods

Botanical Material and Raw Material

Anacardium occidentale Linn. (cashew) stem bark was collected and prepared at the Center for Research in the Semi-Arid Region (NUPEÁRIDO), Federal University of Campina Grande, Patos / PB. The dried specimen was deposited in the "Dárdano de Andrade Lima" Herbarium, Regional University of Cariri (URCA), Crato, Brazil.

When removed, barks were conditioned in plastic bags to prevent loss of moisture. In the laboratory, two samples were collected and cut into smaller fragments, homogenized, weighed and dried in an oven at 103 ± 2°C for 48 hours to determine the moisture content (dry basis). Then, barks were air-dried and ground. The ground material was classified and the material that passed through a 2.00 x 2.00 cm sieve was used. After this operation, four representative bark samples were removed. Two of them were dried at 103 ± 2 ° C for 48 hours to assess the moisture content (dry basis) of bark dried in air and the others were ground in a Willey mill to obtain material with lower grain size and more homogeneous to be used for the quantification of tannic substances present in the species.

The extraction of tannins occurred in water at temperature of 70 ± 5°C for two hours. In extractions, for each 2.00 kg of bark, 10 liters of water were added (5:1 ratio). Each sample was submitted to boiling in a rotary digester with capacity of 20 liters. Each bark sample was submitted to two extractions.

The solution obtained was homogenized and poured into 5 x 40 x 60 cm aluminum trays and placed in a forced air oven maintained at 70 ± 3ºC until complete moisture evaporation. The dried material was milled in a domestic multiprocessor and sieved through a 60-mesh sieve.

Streptococcus mutans ATCC 25175, Streptococcus mitis ATCC 903, Streptococcus sanguis ATCC 15300, Streptococcus oralis ATCC 10557, Streptococcus salivarius ATCC 7073 and Lactobacillus casei ATCC 9595 samples were used, which were obtained upon request at the Fundação Oswaldo Cruz (Rio de Janeiro / RJ) and sent on Blood Agar Slant and then reactivated at the Laboratory of Microbiology, Federal University of Paraíba.

Minimum Inhibitory Concentration (MIC)

The antimicrobial activity (MIC) was obtained using the solid medium diffusion method [14] on Petri dishes. The strains were grown in nutrient broth (BHI - Brain Heart Infusion - DIFCO), incubated at 37°C for 18-20 hours in microaerophilic environment through the candle flame method. Mueller Hinton agar plates (Difco) were prepared and after 24 hours (sterility control), they were flooded with saline and inoculated with microorganisms "overnight" at concentration of 10-2 and then holes of approximately 6 mm in diameter were made. Five holes were prepared in each plate that received numbering ranging from 1 to 10, which corresponded to tannin solution diluted in distilled water (mg / ml) (1:1 up to 1:512). After insertion of 50 μL of test substances, the plates were incubated in bacteriological incubator at 37°C for 24 hours. Each assay

was performed in triplicate for each selected strain. MIC was defined as the lowest substance concentration capable of inhibiting bacterial growth in halos greater than or equal to 10 mm.

Minimum Inhibitory Concentration of Adherence (MICA)

The anti-adherent activity was determined in the presence of 5% sucrose, using increasing and doubled concentrations of diluted solution of isolated tannins ranging from 1:1 to 1:512. From the overnight growth, strains were subcultured at 37°C in Mueller-Hinton broth (DIFCO) to yield an inoculum of 106 CFU / ml. About 1.8 ml of culture were distributed in hemolysis tubes added of 0.2 ml of the solution corresponding to the tannin solution scale. Incubation was carried out at 37°C for 48 hours in microaerophilic environment with tubes inclined at 30º. The reading was performed by visual observation of the adherence of bacteria to the tube walls after stirring. MICA was defined as the lowest tannin concentration in medium with sucrose that prevented the adhesion of microorganisms to the glass tube [15].

For comparative study of both MIC and MICA, 0.12% chlorhexidine gluconate (Periogard®) was used as a positive control. Data were recorded in the database of the SPSS software (Statistical Package for Social Sciences) for Windows® version 15.0 and analyzed using descriptive and inferential statistics.

Results

The results showed that S. mutans, S. mitis, S. sanguis, S. oralis, S. salivarius and L. casei bacterial strains were sensitive to the action of tannins isolated from the cashew bark, showing inhibition halos ranging from 15 to 13 mm, 16 to 11 mm, 17 to 11 mm, 15 to 13 mm, 17 to 13 mm and 15 to 12 mm, respectively, as shown in Table 1. The antimicrobial activity of 0.12% gluconate chlorhexidine was more significant, as shown in Table 2.

Table 1. Minimum Inhibitory Concentration in solid medium of tannins isolated from Anacardium occidentale Linn. (Cashew) on biofilm-forming bacterial strains.

	S mutans	*S mitis*	*S sanguis*	*S oralis*	*S salivarius*	*L casei*
Pure Solution	15mm	16mm	**17mm**	15mm	**17mm**	15mm
1:2	14mm	15mm	14mm	14mm	15mm	12mm
1:4	13mm	14mm	13mm	13mm	14mm	0
1:8	0	13mm	11mm	0	13mm	0
1:16	0	**11mm**	0	0	0	0
1:32	0	0	0	0	0	0
1:64	0	0	0	0	0	0
1:128	0	0	0	0	0	0
1:256	0	0	0	0	0	0
1:512	0	0	0	0	0	0

Table 2. Minimum Inhibitory Concentration in solid medium of 0.12% chlorhexidine gluconate (Periogard®) on biofilm-forming bacterial strains.

	S mutans	*S mitis*	*S sanguis*	*S oralis*	*S salivarius*	*L casei*
Pure Solution	22mm	21mm	**23mm**	22mm	**24mm**	**23mm**
1:2	20mm	20mm	20mm	21mm	22mm	21mm
1:4	19mm	18mm	19mm	20mm	21mm	19mm
1:8	18mm	17mm	17mm	18mm	20mm	17mm
1:16	15mm	14mm	16mm	17mm	19mm	15mm
1:32	13mm	13mm	0	13mm	15mm	0
1:64	**12mm**	0	0	0	0	0
1:128	0	0	0	0	0	0
1:256	0	0	0	0	0	0
1:512	0	0	0	0	0	0

Tannins isolated from Anacardium occidentale Linn. (Cashew) were effective in inhibiting the adherence of the six test strains, represented by the absence of adhesion to glass in the presence of sucrose. The greatest inhibition potential was observed on Streptococcus sanguis and Lactobacillus casei strains. For S. salivarius and S. mitis strains, inhibition of adhesion was observed up to concentration of 1:16 and for S.oralis and S. mutans of 1:8, which can be observed in Table 3.

Table 3. Minimum Inhibitory Concentration of adherence (MICA) in solid medium of tannins isolated from Anacardium occidentale Linn. (Cashew) on biofilm-forming bacterial strains.

Bacterial strains	**Minimum Inhibitory Concentration of adherence**	
	Tannins isolated from *Anacardium occidentale* L.	0.12% chlorhexidine gluconate
S mutans	1:8	1:16
S mitis	1:16	1:16
S sanguis	1:512	1:16
S oralis	1:8	1:16
S salivarius	1:16	1:32
L casei	1:512	1:16

Discussion

Some limitations are related to in vitro experimental studies with planktonic bacteria, since these microorganisms can have their susceptibility to chemical agents changed when they are no longer planktonic and become part of biofilms [16]. However, these studies are important and should not be discarded, since they evaluate the action of antimicrobial agents, indicating whether more complex and expensive studies such as clinical trials, which have greater scientific evidence strength should be performed [17,18].

Previous study [19] evaluated the antimicrobial activity of anacardic acids from Anacardium occidentale L. chestnut bark oil against the following microorganisms: Streptococccus mutans, Staphylococcus aureus, Candida albicans and Candida utilis. Researchers observed that anacardic

acids showed antibacterial activity against all microorganisms, but the greatest inhibitory activity occurred against Streptococcus mutans.

Results from an in vitro study [10] showed that the MIC of extract obtained from Anacardium occidentale L. bark were 12.5 mg / ml for S. mutans and 6.25 mg / ml for S. mitis and S. sanguis. These data are similar to those obtained in the present study with isolated tannins for S. mitis.

The results for the antimicrobial activity of tannins isolated from Anacardium occidentale L. were more effective on S. mitis up to 1:16. The MIC for S. mitis is similar to that obtained in a previous study [20]. In another study, the same bacterial strains used in this study were more sensitive to the action of the extract up to concentrations of 1:16 for S. mutans, 1:8 for S. sanguis and 1:16 for L. casei [20]. It is noteworthy that researchers [20] used crude extract of Anacardium occidentale L. stem bark and not the fraction of tannins isolated as in this study and demonstrated that there are other components in the extract able to inhibit bacterial growth.

Thus, it was observed that both the crude extract as tannins isolated from Anacardium occidentale L have inhibitory activity against microorganisms that are part of the composition of the dental biofilm. Among the hypotheses on the mechanisms of antimicrobial action of tannins, the inhibition of enzymes, the modification of cellular metabolism by its action on membranes and the complexation with metal ions, decreasing their availability to the metabolism of microorganisms stand out [13,21].

Regarding the anti-adherent activity, the comparative study of extract from Anacardium occidentale L bark with 0.12% chlorhexidine solution previously held [20] showed similar activity (1:16) against S. mutans, S. mitis, S. sanguis, S. sobrinus and L. casei strains. In the present work, S. sanguinis and L. casei strains were sensitive at all concentrations evaluated, i.e. up to 1:512. These data demonstrate that the in vitro anti-adherent activity of Anacardium occidentale L bark may be strongly associated with the presence of tannins, which are phenolic compounds present in the extract of this plant with proven antimicrobial and anti-adherent activity.

All tested strains were susceptible to the antimicrobial and anti-adherent activity of 0.12% chlorhexidine gluconate and corroborates previous studies that evaluated the effect of this substance against the growth of microorganisms forming dental biofilm [17,22].

In vitro results are promising to evaluate these compounds in the form of mouthwash; in in vivo studies, aiding mechanical methods of oral hygiene. Reports of the anti-adherent activity of tannins isolated from cashew bark on biofilm microorganisms are rare or nonexistent.Therefore, in groups in which there was a mixture of chocolate and coffee to the milk, a smaller reduction in microhardness (compared to orange juice), with values that did not differ from milk was found, suggesting that the addition of these components to milk does not interfere in its protective capacity and confirming the results found in the literature in relation to chocolate milk [13] and compared to pure coffee [23].

Within the limitations of this in vitro study, the findings have clinical importance. Other methods of erosion control such as application of fluoride varnish and fluoride gel would depend on the cooperation of the patient going the dental office every period of time for the application to be made, implying a cost to this type of treatment. The prevention of erosion lesions can be achieved more easily and can be more widespread by giving instructions and encouraging patients to the milk intake, whether or not with any additive such as chocolate or coffee after the consumption of soft drinks, juices of acidic fruits or just fruits. It is a type of food that is part of the routine of the population and which has been incorporated into the dietary habits and it can help to stabilize the progression of loss of tooth structure. However, more studies proving the efficacy of milk intake should be conducted, with some in situ or in vivo model, so that it is possible to simulate the real oral conditions, such as saliva and brushing.

Conclusion

Tannins isolated from Anacardium occidentale Linn. (cashew) stem bark showed in vitro antimicrobial and anti-adherent activity against biofilm-forming bacterial strains. Thus, tannic solution can be considered as an alternative treatment for oral diseases, especially those caused by the presence of biofilm such as caries and periodontal disease.

References

1. Oppermann, RV, Haas AN, Villoria GEM, Primo LG, Serra-Negra JM, Ferreira EF, Pannuti CM. Proposal for the teaching of the chemical control of supragingival biofilm. Braz Oral Res. 2010; 24(Suppl 1):33-6.
2. Nogueira-Filho GR, Toledo S, Cury JA. Effect of 3 dentifrices containing triclosan and various additives. J Clin Periodontol 2000; 27(7):494-8.
3. Pradeep AR, Agarwal E, Naik SB. Clinical and Microbiologic Effects of Commercially Available Dentifrice Containing Aloe Vera: A Randomized Controlled Clinical Trial. J Periodontol 2012; 83(6):797-804.
4. Lawrence JR, Zhu B, Swerhone GDW, Topp E, Roy J, Wassenaar LI, Rema T, Korber DR. Community-level assessment of the effects of the broad-spctrum antimicrobial chlorhexidine on the outcome of river microbial biofilm development. Appl Environ Microbiol. 2008; 17(74):3541-50.
5. Botelho MA, Nogueira NAP, Bastos GM, Fonseca SGC, Lemos TLG, Matos FJA, Montenegro D, Heukelbach J, Rao VS, Brito GA. Antimicrobial activity of the essential oil from Lippia sidoides, carvacrol and thymol against oral pathogens. Braz J Med Biol Res 2007; 40(3):349-56.
6. Chaves MH, Citó AMGL, Lopes JAD, Costa DA, Oliveira CAA, Costa AF, Brito- Júnior, FEM. Fenóis totais, atividade antioxidante e constituintes químicos de extratos de Anacardium occidentale L., Anacardiaceae. R Bras Farmacogn 2010; 20(1):106-12.
7. Morais TC, Pinto NB, Carvalho KM, Rios JB, Ricardo NM, Trevisan MT, Rao VS, Santos FA. Protective effect of anacardic acids from cashew (Anacardium occidentale) on ethanol-induced gastric damage in mice. Chem Biol Interact 2010; 183(1):264-69.
8. Silva JG, Souza IA, Higino JS, Siqueira-Junior JP, Pereira JV, Pereira MSV. Atividade antimicrobiana do extrato de Anacardium occidentale Linn. em amostras multiresistentes de Staphylococcus aureus. R Bras Farmacogn 2007; 17(4):572-77.
9. Tchikaya FO, Bantsielé GB, Kouakou-Siransy G, Datté JY, Yapo PA, Zirihi NG, Offoumou MA. Anacardium occidentale linn. (anacardiaceae) stem bark extract induces hypotensive and cardio-inhibitory effects in experimental animal models. Afr J Tradit Complement Altern Med 2011; 8(4):452-61.
10. Melo AFM, Santos EJV, Souza LFC, Carvalho AAT, Pereira MSV, Higino JS. Atividade antimicrobiana in vitro do extrato de Anacardium occidentale L. sobre espécies de Streptococcus. R Bras Farmacogn 2006; 16(2):202-5.

11. Araújo CRF, Pereira JV, Pereira MSV, Alves PM, Higino JS, Martins AB. Concentração Mínima Bactericida do Extrato do Cajueiro sobre Bactérias do Biofilme Dental. Pesq Bras Odontoped Clin Integr 2009; 9(2):187-91.
12. Haslam E. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. J Nat Prod. 1996; 59(2):205-15. 13. Scalbert A. Antimicrobial properties of tannins. Phytochemistry 1991; 12(30):3875-83.
14. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45(4):493-96.
15. Gebara ECE, Zardetto CGDC, Mayer MPA. Estudo in vitro da ação antimicrobiana de substâncias naturais sobre S. mutans e S. sobrinus. Rev Odontol Univ São Paulo 1996; 4(10):251-65.
16. Pan P, Barnett ML, Coelho J, Brogdon C, Finnegan MB: Determination of the in situ bactericidal activity of an essential oil mouthrinse using a vital stain method. J Clin Periodontol 2000; 27:256-61.
17. Macêdo-Costa MR, Pereira MSV, Pereira LF, Pereira AV, Rodriques OG. Atividade Antimicrobiana e Antiaderente do Extrato da Mimosa tenuiflora (Willd). Poir. Sobre Microrganismos do Biofilme Dentário. Pesq Bras Odontoped Clin Integr 2009; 9(2):161-5.
18. Primo BT, Grazziotin-Soares R, Bertuzzi D, Claudy MP, Hernandez PAG, Fontanella VRC. Produção científica da ULBRA: análise do número e do delineamento das pesquisas publicadas nos suplementos da Brazilian Oral Research (SBPqO). Stomatos 2010; 16(31):69-6.
19. Lima CAA, Pastore GM, Lima EDPA. Estudo da atividade antimicrobiana dos ácidos anacárdicos do óleo da casca da castanha de caju (CNSL) dos clones de cajueiro – anão-precoce CCP – 76 e CCp 09 em cinco estágios de maturação sobre microrganismos da cavidade bucal. Ciênc Tecnol Aliment 2000; 20(3):358-62.
20. Araújo CRF. Estudo da ação antimicrobiana e antifúngica do extrato do citrus limon linn. (limão) e do anacardium occidentale linn. (cajueiro) sobre microrganismos do biofilme dental e leveduras do gênero Candida. [Dissertação]. João Pessoa: Universidade Federal da Paraíba; 2005.
21. Monteiro JM, Albuquerque UP, Araujo EL, Amorim ELC. Taninos: uma abordagem da química à ecologia. Quím. Nova 2005, 28(5):892-6.
22. Alves PM, Pereira JV, Higino JS, Pereira MSV, Queiroz LMG. Atividade antimicrobiana e antiaderente in vitro do extrato de rosmarinus officinalis linn. (alecrim) sobre microrganismos cariogênicos. Arq Odontol 2008; 44(2):53-8.