Revista Brasileira de Biociências
Brazilian Journal of Biosciences

ISSN 1980-4849 (on-line) / 1679-2343 (print)

ARTICLE

Effect of cashew (*Anacardium occidentale* L.) peduncle bagasse extract on *Streptococcus mutans* and its biofilm

Manuela Almeida Montenegro Furtado[1], Francisca das Chagas de Sousa Alves[1], Jorge Luiz Martins[2], Mayron Alves de Vasconcelos[3], Vanessa Sabrine Correia Ramos[1], Giselly Soares de Sousa[1], André Luis Coelho da Silva[3], Wladimir Ronald Lobo Farias[5], Edson Holanda Teixeira[4], Benildo Sousa Cavada[3] and Ricardo Pires dos Santos[1,*]

Received: August 31 2013 Received after review: November 22 2013 Accepted: February 19 2014
Available online at http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/2750

ABSTRACT: (Effect of cashew (*Anacardium occidentale* L.) peduncle bagasse extract on *Streptococcus mutans* and its biofilm). This study investigated the antimicrobial activity of cashew (*Anacardium occidentale* L.) peduncle bagasse extract. Cashew peduncle bagasse extract was prepared, and its minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), and effect on biofilm formation were determined against the strain *Streptococcus mutans* UA159. The bagasse extract showed MIC at a concentration of 500 µg/mL and MBC at 1000 µg/mL against *S. mutans*. At 250 µg/mL, the extract significantly reduced the biofilm formation of *S. mutans,* possibly associated with its effect on planktonic cells. The results of this study showed that cashew peduncle residue has biomedical potential as an antimicrobial agent against an important pathogen responsible for the formation of biofilm and subsequent dental caries.
Key words: *Anacardium occidentale*, biofilm, extract, peduncle, residue, *Streptococcus mutans*.

RESUMO: (Efeito do extrato do bagasso do pedúnculo do caju (*Anacardium occidentale* L.) sobre *Streptococcus mutans* e seu biofilme). Este estudo investigou a atividade antimicrobiana do extrato do bagaço do pedúnculo do caju (*Anacardium occidentale* L.). O extrato do bagasse do caju foi preparado e sua concentração inibitória mínima (CIM), concentração bactericida mínima (CBM) e seu efeito sobre a formação de biofilme foram determinadas sobre a estirpe *Streptococcus mutans* AU 159. O extrato do bagaço apresentou CIM a uma concentração de 500 µg/mL e CBM a 1000 µg/mL contra *S. mutans*. A 250 µg/mL, o extrato reduziu significantemente a formação de biofilme de *S. mutans*, possivelmente associado ao seu efeito sobre células planctônicas. Os resultados desse estudo mostraram que o resíduo do pedúnculo do caju tem potencial biomédico como agente antimicrobiano contra um importante patógeno responsável pela formação de biofilme e subsequentemente carie dental.
Palavras-chave: *Anacardium occidentale*, biofilme, extrato, pedúnculo, resíduo, *Streptococcus mutans*.

INTRODUCTION

The cashew tree (*Anacardium occidentale* L.) occupies an important position among tropical fruit trees, with a growing market for its main products, including the nut and the peduncle, also known as the cashew "apple" (Leitão *et al*. 2013). According to De Abreu *et al*. (2013), in Brazil the average annual production of cashew is 260 000 tons, which corresponds to a production of over 2 million tons of cashew peduncle. The peduncle is processed and used for the production of drinks, sweets, jams, nectar, flour, fermented products and animal feed, but less than 6% of the peduncle produced is presently used (Moura 1998, Paiva & Barros 2004, De Abreu *et al*. 2013). The complex chemical composition of the peduncle includes proteins, sugars, tannins, flavonoids, ascorbic acid, phosphorous, calcium, iron, and vitamins A and C (FAO 1986, Rodriguez-Amaya *et al*. 2009, Queiroz *et al*. 2011). Its juice has been used to produce a film with healing, analgesic and anti-inflammatory properties (Silva 2002). Moreover, peduncle bagasse ash has shown antifungal properties against important species of the genus *Fusarium,* as a result of the large amounts of $KHCO_3$ produced (Karabulut *et al*. 2006, Diliopoulos *et al*. 2010, Santos *et al*. 2011).

Cashew peduncle bagasse, a by-product of the juice extraction process, comprises approximately 20% of the total weight of the peduncle (AGRIANUAL 2000, da Costa *et al*. 2009). It is one of the largest sources of residues (90–94%) produced by the cashew industry, and is presently used only as a nutritional supplement in animal feed (Santos *et al*. 2007, da Costa *et al*. 2009). This limited use is a result of its rapid degradation, which makes it impossible to store.

Organic-solvent extracts from aerial parts, bark, flowers, fruits, heartwood, leaves, twigs and roots from plants have been investigated for their ethnopharmacological use (Paiva *et al*. 2010). Cashew extracts have been reported to possess several pharmacological properties, including anti-inflammatory, antioxidant, antitumor, and antimicrobial against pathogenic microorganisms

1. Laboratório de Engenharia de Computação e Materiais de Sobral (LEMCS), Universidade Federal do Ceará, Sobral, CE, Brazil.
2. Instituto de Química e Biosciências, Universidade Federal de Pelotas, Pelotas, RS, Brazil.
3. Laboratório de Moléculas Biologicamente Ativas (BioMol-Lab), Universidade Federal do Ceará, Fortaleza, CE, Brazil.
4. Laboratório Integrado de Biomoléculas (LIBS), Universidade Federal do Ceará, Departamento de Patologia e Medicina Legal, Faculdade de Medicina, Fortaleza, CE, Brazil.
5. Departamento de Engenharia de Pesca, Universidade Federal do Ceará, Fortaleza, CE, Brazil.
* Author for correspondence. E-mail: rpsantos2007@gmail.com

(Broinizi *et al*. 2007, Carvalho *et al*. 2011, Pereira *et al.* 2011, Onasanwo *et al*. 2012, Gonçalves & Gobbo 2012). Pathogenic bacteria rarely exist in the form of pure planktonic cultures (Santos *et al*. 2008); instead, they adhere to surfaces, and thus persist within a highly complex ecosystem comprised of a structured biofilm established inside the host. Bacteria in a biofilm are more resistant to the action of antibacterial agents (Cataldi *et al*. 2013). Dental plaque is a type of biofilm that develops on the surfaces of teeth and is a precursor to tooth decay. Oral biofilm consists of multiple species of bacteria that take part in the complex ecosystems of the oral cavity. One such species is *Streptococcus mutans*, a gram-positive bacterium that is the primary causative agent of dental caries (Takahashi & Nyvad 2011, Bedran *et al*. 2013). Several products originating directly from plants, such as those in the form of extracts and infusions, have shown activity against microorganisms related to dental caries (Naidoo *et al*. 2012). Other studies report that stem bark and leaves of cashew show antibacterial activity against many biofilm-forming oral bacteria (Kudi *et al*. 1999, da Silva *et al*. 2007, Araújo *et al*. 2009).

This study evaluated the antimicrobial activity of cashew peduncle bagasse extract against planktonic cultures and biofilm formation by *Streptococcus mutans*.

MATERIALS AND METHODS

Production of extract

Samples of red cashew peduncles were obtained from plantations in the State of Ceará, Brazil during September and October 2008. After the nut was extracted, the peduncles were pressed to remove the juice, producing the cashew bagasse. The bagasse was then dried in an oven at 36 ºC for 48 h and ground in an analytical mill. For production of the extract, the bagasse powder was added to a 70% ethanol solution, stirred for 30 min, and stored at room temperature for 7 days. The solution was filtered and subjected to rotary evaporation in a water bath at 60 ºC for 48 h. The extract was lyophilized for 24 h to form a powder that was reconstituted in 0.9% NaCl at 2000 µg/mL and stored for later use.

Microorganism and culture conditions

The bacterial strain *Streptococcus mutans* UA 159 used in this study was obtained from the Instituto Oswaldo Cruz (FIOCRUZ) collection. The strain was kept in BHI (Brain Heart Infusion; Difco) and 20% glycerol at −80 °C. For the experimental procedures, 100 µL of the medium containing bacteria was inoculated into 10 mL of fresh BHI broth and incubated for 18 h at 37 °C in 10% CO_2. After initial activation, the culture was renewed using 100 µL of inoculum in 10 mL of sterile BHI broth and grown under the same conditions as described above.

Antimicrobial assays

The effects of the extract on bacterial growth (planktonic culture) were determined using the broth microdilution method in 96-well polystyrene plates, according to the procedure outlined by the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS 2003). After cell growth, the culture was centrifuged at 1500 g for 20 min at 4 °C, washed twice with 0.9% NaCl and then adjusted to a concentration of 10^8–10^9 cells/mL in BHI broth. The extract solutions were prepared by 2-fold serial dilutions in 0.9% NaCl, with final concentrations of 7.8, 15.6, 31.25, 62.5, 125, 250, 500 and 1000 µ/mL. The assay in polystyrene plates was carried out by the addition of 100 µL of bacteria (10^8–10^9 cells/mL) in BHI broth to each well and 100 µL of the extract solution at each concentration. The negative control used was 0.9% NaCl, which represents the normal growth of bacteria using the same procedure as for the bacteria/extract. Chlorhexidine (0.12%) was used as a positive control. The optical density at 640 nm of the contents of each well was recorded using an automated Elisa Reader (Synergy™ HT Multi-Mode Microplate Reader, Bio-Tek Instruments, Winooski, VT, USA) to measure microbial growth. When the plates were ready, an initial measurement was carried out to establish a baseline. Measurements were made after 24 h of incubation at 37 °C in 10% CO_2. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of extract that prevented visible growth of the microorganism. The minimal bactericidal concentration (MBC) was evaluated as described by Sá *et al*. (2012). To determine the MBC, 50 µL of bacterial suspension from the wells corresponding to each concentration tested was inoculated in 5 mL of sterile BHI broth medium and incubated for 24 h at 37 ºC in 10% CO_2. MBC was considered the lowest concentration that completely inhibited bacterial growth in the medium.

Effect of peduncle bagasse extract on biofilm formation

The methodology used to monitor biofilm growth was based on the microtiter plate test developed by Stepanovic *et al*. (2000) with some modifications. Briefly, sterile 96-well flat-bottom polypropylene tissue-culture plates were prepared using a procedure similar to that used to assay the effects on bacterial growth, with the same initial concentration of cells. Biofilms were developed in microtiter plate wells containing BHI broth supplemented with 1% sucrose at 37 °C in 10% CO_2 for 24 h. Biofilm growth was tested in the presence of extract, at the final concentrations of 15.6, 31.25, 62.5, 125, 250 and 500 µ/mL, 0.9% NaCl, or 0.12% chlorhexidine for 24 h. Following incubation, the contents of each well was removed and the biofilms were washed twice with 200 µL/well of sterilized water to remove weakly adherent cells. These cells were preserved for later analysis. The attached biofilm mass was quantified using crystal violet staining (Burton *et al*. 2007). Briefly, the plates containing the biofilms were dried in air for 30 min, and

200 µL of absolute methanol was transferred to each well to fix the adherent cells. After 15 min, the methanol was removed, and 200 µL of 1% crystal violet (Gram color-staining set for microscopy; Merck, Darmstadt, Germany) per well was added. After 5 min, the washing process was repeated with sterile water, and the plates were allowed to stand at room temperature for 1 h. To solubilize the dye bound to biofilms, 200 µL of 33% (v/v) glacial acetic acid (Merck) was added to each well, and the plates were agitated for 15 min. The resulting solutions were transferred to a new sterile 96-well plate, and the optical densities at 570 nm for the contents of each well were recorded using an automated Elisa Reader (Synergy HT Multidetection Microplate Reader, Bio-Tek Instruments).

Statistical analysis

The results from nine repetitions (n = 9) of at least three separate experiments were obtained. Quantitative data are expressed as the means ± standard deviation (SD). A Mann-Whitney non-parametric test was performed to determine the significant differences between the groups (Triola 2008). The significance level used was 0.01 ($P < 0.01$). All statistical analyses were performed using OriginPro 8.5 software (OriginLab Corporation, Northhampton, MA, USA).

RESULTS AND DISCUSSION

As shown in figure 1, peduncle bagasse extract was able to inhibit the planktonic growth of *S. mutans,* with a MIC value of 500 µg/mL; and at a concentration of 250 µg/mL the extract reduced planktonic growth by approximately 96% relative to the absorbance of the negative control (NaCl). The chlorhexidine solution (positive control), a powerful antimicrobial agent used in dentistry, inhibited bacterial growth compared to the negative control. At 1000 µg/mL, the extract killed all the bacteria (MBC).

Figure 2 shows the effects of the peduncle bagasse extract on biofilm growth. At 500 and 250 µg/mL, the extract reduced the biofilm formation. As expected, chlorhexidine inhibited the biofilm formation compared to the negative control. Extract solutions with concentrations equal to or greater than 250 µg/mL inhibited biofilm growth. At these concentrations, the extract solution reduced the biofilm formation approximately 96% compared to the absorbance of the negative control.

The causal factors that underlie the antimicrobial effect of peduncle bagasse extract require additional investigation. To accomplish this, it is necessary to study the chemical and phase composition. According to Andrade (2013), cashew peduncle bagasse extract contains several bioactive phytochemicals, including the major compounds carotenoids, ascorbic acid, flavonoids and tannins. One or more of these compounds, acting together or separately, may be responsible for the high efficacy of this extract against the growth of bacteria and biofilm formation. Several studies have described antimicrobial activity of extracts from different parts of *A. occidentale*, including the bark, leaves, nuts and peduncle (Arekemase *et al*. 2011, Lasca & Gonçalves 2012, Gonçalves & Gobbo 2012). Lasca and Gonçalves (2012) showed that the ethanolic extract of the peduncle inhibits the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Candida albicans*, and suggested that this antibacterial activity of the extract is due to the presence of tannins. Green *et al*. (2008) observed that anacardic acids extracted from peduncle juice exhibit antimicrobial activity against *S. mutans* at a concentration of 800 µg/mL. Considering the diversity of constituents present in the cashew-peduncle extract, the antibacterial activity of the extract against *S. mutans* may be due to the presence of phenolic compounds such as tannins, flavonoids or others. The mechanisms responsible for the antimicrobial activity of phenolic compounds present in plant extracts are not fully understood; however, several studies have

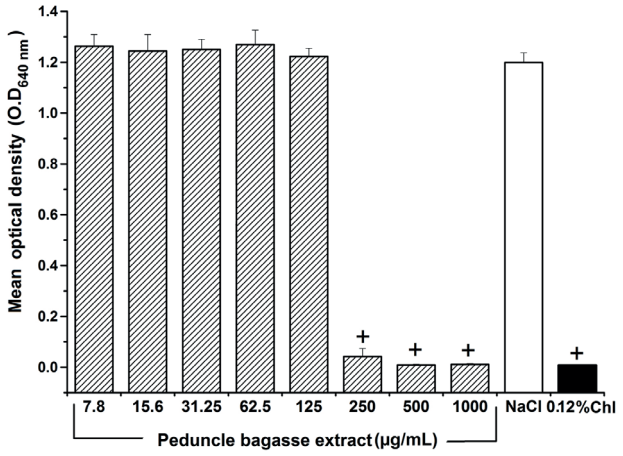

Figure 1. The antibacterial activity of peduncle bagasse extract against *Streptococcus mutans* UA159. (+) P<0.01 compared to negative control (NaCl).

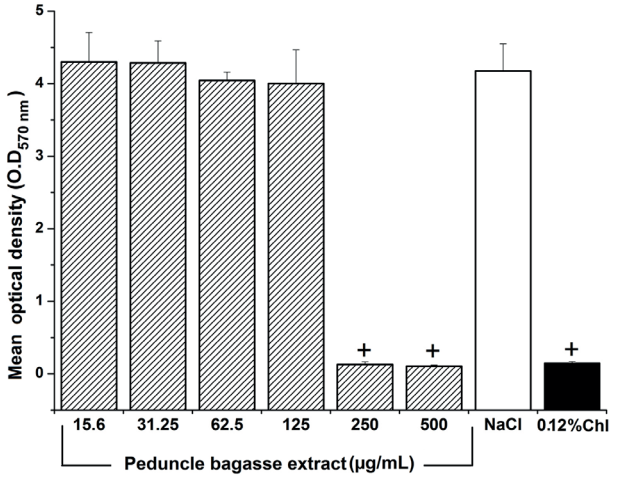

Figure 2. The effect of peduncle bagasse extract on the biofilm formation of *Streptococcus mutans* UA159. (+) P<0.01 compared to negative control (NaCl).

suggested that these compounds may inactivate important enzymes and damage the cytoplasmic membrane (Paiva *et al*. 2010, Cushnie & Lamb 2011, Nitiema *et al*. 2012, Alves *et al*. 2013).

The oral cavity contains several microenvironments that can be colonized by bacteria and consequently support the formation of biofilm. Biofilms are involved in the etiology of the most common diseases of the oral cavity, such as dental caries and periodontal disease (Martins *et al*. 2012). *Streptococcus mutans* is commonly found in the human oral cavity and contributes significantly to tooth decay (Biswas *et al*. 2010). In the present study, an extract from cashew peduncle bagasse showed bacteriostatic and bactericidal activity on *S. mutans*, and inhibited biofilm formation. Probably, the reduction of the biofilm formation by the extract from cashew peduncle occurs due to its bacteriostatic and bactericidal activity. These findings are consistent with those of Araújo *et al*. (2009), who demonstrated that the hydroalcoholic extract of stem bark from *A. occidentale* showed bactericidal activity against important biofilm-forming bacteria, including *S. mutans*. Thus, the antimicrobial properties of the peduncle bagasse extract show potential for its use in oral health, supporting the antimicrobial activity of oral antiseptics.

The bagasse from cashew peduncle is an industrial residue remaining after juice extraction, and its use is rather limited (Broinizi *et al*. 2007). The industrial residues cause potential environmental problems, resulting in losses of raw materials and energy, and hence requiring significant investments in treatments to control pollution (Pelizer *et al*. 2007, Marques 2013). On the other hand, studies have shown the presence of bioactive compounds in different types of agro-industrial residues, with potentially valuable applications in industry (Martin *et al*. 2012). Moreover, residues of fruits can be used as a source of raw material to identify antimicrobial agents. Studies have found bioactive compounds with antimicrobial activity in the bagasse of grapes (Adámez *et al*. 2012), pomegranate peels (Al-Zoreky 2009) and lemon rinds (Mahmud *et al*. 2009). Thus, one alternative use for cashew peduncle bagasse might be as an antimicrobial agent in the prevention and control of caries.

In this study, bagasse from the peduncle of cashew (*Anacardium occidentale* L.), a residue of the Brazilian cashew agribusiness, showed antibacterial activity against *Streptococcus mutans*. These results suggest that peduncle bagasse extract has a potential use as a prophylaxis for dental caries; however, toxicological studies will need to be performed to validate its applicability in this regard.

ACKNOWLEDGMENTS

The authors thank the professors, technicians and students of the Biotechnology Center of Sobral (NUBIS), and the Biologically Active Molecules Laboratory (BioMol-Lab) of the Federal University of Ceará, Ceará, Brazil for research facilities, technical support and important suggestions during this study. B. S. Cavada and E. H. Teixeira are investigators for the National Council of Research (CNPq). The study received partial financial support from CNPq - Project numbers 501221/2009-3 and 473911/2010-8. AJE (American Journal Experts) and Janet W. Reid (JWR Associates) helped with the English editing of the manuscript.

REFERENCES

ADÁMEZ, J.D., SAMINO, E.G., SÁNCHEZ, E.V. & GONZÁLEZ-GÓMEZ, D. 2012. *In vitro* estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape-seeds (*Vitis vinifera* L.). *Food Control, 24*: 136-141.

AGRIANUAL. 2000. Anuário da Agricultura Brasileira. São Paulo: FNP Consultoria & Comércio. p. 244-248.

ALVES, M.J., FERREIRA, I.C., FROUFE, H.J., ABREU, R.M., MARTINS, A. & PINTADO, M. 2013. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. *J Appl Microbiol.*, *115*(2): 346-57.

AL-ZOREKY, N.S. 2009. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. *Int J Food Microbiol., 134*: 244-248.

ANDRADE, R.A.M.A. 2013. *Fitoquímicos bioativos e potencial antioxidante do resíduo agroindustrial do pedúnculo do caju (Anacardium occidentale L.).* 95 p. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos). Universidade Federal Rural de Pernambuco, Recife, 2013.

ARAÚJO, C.R.F., PEREIRA, J.V., PEREIRA, M.S.V., ALVES, P.M., HIGINO, J.S. & MARTINS, A.B. 2009. Bactericidal minimum concentration of extract of popularily known as "cajueiro" upon bacterias of dental biofilm. *Pesq Bras Odontoped Clin Integr., 9*(2): 187-191.

AREKEMASE, M.O., OYEYIOLA, G.P. & ALIYU, M.B. 2011. Antibacterial activity of *Anacaridum occidentale* on some enterotoxin producing bactéria. *Int J Biol*., *3*(4); 1-13.

BEDRAN, T.B.L., AZELMAT, J., SPOLIDORIO, D.P. & GRENIER, D. 2013. Fibrinogen-induced *Streptococcus mutans* biofilm formation and adherence to endothelial cells. *Biomed Res Int*., *2013*: 1-8.

BISWAS, S., BOWLER, I.C.J.W., BUNCH, C., PRENDERGAST, B. & WEBSTER, D.P. 2010. *Streptococcus mutans* infective endocarditis complicated by vertebral discitis following dental treatment without antibiotic prophylaxis. *J Med Microbiol., 59*: 1257-1259.

BROINIZI, P.R.B., ANDRADE-WARTHA, E.R.S., SILVA, A.M.O., NOVOA, A.J.V., TORRES, R.P., AZEREDO, H.M.C., ALVES, R.E. & MANCINI-FILHO, J. 2007. Evaluation of the antioxidant activity of phenolic compounds naturally contained in by-products of the cashew apple (*Anacardium occidentale* L.). *Ciênc. Tecnol. Aliment., 27*(4): 902-908.

BURTON, E., YAKANDAWALA, N., LOVETRI, K. & MADHYASTHA, M.S. 2007. A microplate spectrofluorometric assay for bacterial biofilms. *J. Ind. Microbiol. Biotechnol., 34*: 1-4.

CATALDI, M., SBLENDORIO, V., LEO, A. & PIAZZA, O. 2013. Biofilm-dependent airway infections: A role for ambroxol? *Pulm Pharmacol Ther*. (in press).

CARVALHO, A.L., ANNONI. R., SILVA, P.R., BORELLI, P., FOCK, R.A., TREVISAN, M.T. & MAUAD, T.J. 2011. Acute, subacute toxicity and mutagenic effects of anacardic acids from cashew (*Anacardium occidentale* Linn.) in mice. *Ethnopharmacology*, *135*(3): 730-736.

CUSHNIE, T.P.T. & LAMB, A.J. 2011. Recent advances in understanding the antibacterial properties of flavonoids. *Int J Antimicrob Ag*., *38*: 99-107.

DA COSTA, J.M.R., FELIPE, E.M.F., MAIA, G.A., HERNANDEZ, ISABELLA. & BRASIL, F.F.F.M. 2009. Production and characterization of the cashew apple (*Anacardium occidentale* L.) and guava (*Psidium guajava* L.) fruit powders. *J. Food Process. Preserv*., *33*: 299-312.

DA SILVA, J.G., SOUZA, I.A., HIGINO, J.S., SIQUEIRA-JUNIOR, J.P., PEREIRA, J.V. & PEREIRA, M.S.V. 2007. Atividade antimicrobiana do extrato de *Anacardium occidentale* Linn. em amostras multiresistentes de *Staphylococcus aureus*. *Rev. Bras. Farmacogn*., *17*(4): 572-577.

DE ABREU, F.P., DORNIER, M., DIONISIO, A.P., CARAIL, M., CARIS-VEYRAT, C. & DHUIQUE-MAYER, C. 2013. Cashew apple (*Anacardium occidentale* L.) extract from by-product of juice processing: a focus on carotenoids. *Food Chem*., *138*(1): 25-31.

DILIOPOULOS, T., KETTLEWELL, P.S. & MARTIN, C.H. 2010. Fungal disease suppression by inorganic salts: A review. *Crop Protection, 29*: 1059-1075.

FOOD AND AGRICULTURAL ORGANIZATION. 1986. *Food and fruitbearing forest species. 3. Examples from Latin America*. Rome. p. 19-23.

GONÇALVES, G.M.S & GOBBO, J. 2012. Antimicrobial effect of *Anacardium occidentale* extract and cosmetic formulation development. *Braz. arch. biol. technol*., *55*(6): 843-850.

GREEN, I.R., TOCOLI, F.E., LEE, S.H., NIHEI, K. & KUBO, I. 2008. Design and evaluation of anacardic acid derivatives as anticavity agents. *Eur J Med Chem., 43*: 1315-1320.

KARABULUT, O.A., ARSLAN, U., ILHAN, K. & YAGDI, K. 2006. The effect of sodium bicarbonate alone or in combination with a reduced rate of mancozeb on the control of leaf rust *Puccinia triticina* in wheat. *Can. J. Plant Pathol., 28*: 484-488.

KUDI, A.C., UMOH, J.U., EDUVIE, L.O. & GEFU, J. 1999. Screening of some Nigerian medicinal plants for antibacterial activity. *J Ethnopharmacol., 67*: 225-228.

LASCA, T.B. & GONÇALVES, G.M.S. Avaliação da eficácia antimicrobiana de formulações contendo extrato de *Anacardium occidentale*. In: II Encontro de Iniciação e Desenvolvimento Tecnológico e Inovação, 2012, Campinas. *Anais do II Encontro de Iniciação e Desenvolvimento Tecnológico e Inovação*. Ed. da PUC, Campinas.

LEITÃO, N.C.M.C.S., PRADO, G.H.C., VEGGI, P.C., MEIRELES, M.A.A. & PEREIRA, C.G. 2013. *Anacardium occidentale* L. leaves extraction via SFE: Global yields, extraction kinetics, mathematical modeling and economic evaluation. *J. Supercrit Fluids*, *78*: 114–123.

MAHMUD, S., SALEEM, M., SIDDIQUE, S., AHMED, R., KHANUM, R. & PERVEEN, Z. 2009. Volatile components, antioxidant and antimicrobial activity of *Citrus acida* var. sour lime peel oil. *J Saudi Chem Soc., 13*: 195-198.

MARTIN, J.G.P., PORTO, E., CORRÊA, C.B., ALENCAR, S.M., GLORIA, E.M., CABRAL, I.S.R. & AQUINO, L.M. 2012. Antimicrobial potential and chemical composition of agro-industrial wastes. *J Nat Prod., 5*: 27-36.

MARTINS, M.; MONTEIRO, D.R. & OLIVEIRA, R. 2012. Biofilmes orais. In: AZEVEDO, N.F. & CERCA, N. (Eds.) *Biofilmes - Na Saúde, no Ambiente, na Indústria*. Porto: Tipografia Lousanense. p. 1-12.

MARQUES, T.R. 2013. *Aproveitamento tecnológico de resíduos de acerola: farinhas e barras de cereais*. 101 p. Dissertação (Mestrado em Agroquímica). Universidade Federal de Lavras, Lavras, 2013.

MOURA, C.F.H. 1998. *Qualidade de pedúnculos de clones de cajueiro-anão-precoce (Anarcadium occidentale L. var.* nanum*) irrigados*. Dissertação de Mestrado, Universidade Federal do Ceará, Ceará, Fortaleza, 1998.

NAIDOO, R., PATEL, M., GULUB, Z. & FENYVESI, I. 2012. Inhibitory activity of *Dodonaea viscosa var. angustifolia* extract against *Streptococcus mutans* and its biofilm. *J Ethnopharmacol., 144*: 171-174.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS. 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard – Seventh Edition CLSI M7-A7. Clinical and Laboratory Standards Institute: Wayne, Pennsylvania.

NITIEMA, L.W., SAVADOGO, A., SIMPORE, J., DIANOU, D. & TRAORE, A.S. 2012. In vitro antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains. *Intl. J. Microbiol. Res., 3*(3): 183-187.

ONASANWO, S.A., FABIYI, T.D., OLUWOLE, F.S. & OLALEYE, S. B. 2012. Analgesic and anti-inflammatory properties of the leaf extracts of *Anacardium occidentalis* in the laboratory rodents. *Niger J Physiol Sci., 27*(1): 65-71.

PAIVA, J. R. & BARROS, L. M. 2004. *Clones de cajueiro: obtenção, características e perspectivas.* Fortaleza: Embrapa Agroindústria Tropical.

PAIVA, P.M.G., GOMES, F.S., NAPOLEÃO, T.H., SÁ, R.A., CORREIA, M.T.S. & COELHO, L.C.B.B. 2010. *Antimicrobial activity of secondary metabolites and lectins from plants.* Badajoz: Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. p. 396-406.

PELIZER, L.H., PONTIERI, M.L., MORAES, I.O. 2007. Utilização de resíduos agro-industriais em processos biotecnológicos como perspectiva de redução do impacto ambiental. *J. Technol. Manag. Innov*., *2*(1): 118-127.

PEREIRA, E.M, GOMES, R.T., FREIRE, N.R., AGUIAR, E.G., BRANDÃO, M.D. & SANTOS, V.R. 2011. In vitro antimicrobial activity of Brazilian medicinal plant extracts against pathogenic microorganisms of interest to dentistry. *Planta Med*., *77*(4): 401-404.

QUEIROZ, C., LOPES, M.L.M., FIALHO, E. & VALENTE-MESQUITA, V.L. 2011. Changes in bioactive compounds and antioxidant capacity of fresh-cut cashew apple. *Food Res. Int*., 44(5): 1459-1462.

RODRIGUEZ-AMAYA, D. B., KIMURA. M. & AMAYA-FARFAN, J. 2009. Fontes brasileiras de carotenóides. Tabela brasileira de composição de carotenóides em alimentos. Brasilia: Ministério do Meio Ambiente.

SÁ, N.C., CAVALCANTE, T.T.A., ARAÚJO, A.X., SANTOS, H.S., ALBUQUERQUE, M.R.J.R., BANDEIRA, P.N., CUNHA, R.M.S., CAVADA, B.S. & TEIXEIRA, E.H. 2012. Antimicrobial and antibiofilm action of casbane diterpene from *Croton nepetaefolius* against oral bacteria. *Arch. Oral Biol*., *57*(5): 550-555.

SANTOS, R.P., SANTIAGO, A.A.X., GADELHA, C.A.A., CAJAZEIRAS, J.B., CAVADA, B.S., MARTINS, J.L., OLIVEIRA, T.M., BEZERRA, G.A., SANTOS, R.P. & FREIRE, V.N. 2007 Production and characterization of the cashew (*Anacardium occidentale* L.) peduncle bagasse ashes. *J Food Eng., 79*: 1432-1437.

SANTOS, R.P., ARRUDA, T.T.P., CARVALHO, C.B.M., CARNEIRO, V.A., BRAGA, L.Q.V., TEIXEIRA, E.H. ARRUDA, F.V.S., CAVADA, B.S., HAVT, A., DE OLIVEIRA, T.M., BEZERRA, G.A. & FREIRE, V.N. 2008. Correlation between *Enterococcus faecalis* biofilms development stage and quantitative surface roughness using atomic force microscopy. *Microsc. Microanal., 14*: 1-9.

SANTOS, R.P., MARINHO, M.M., SÁ, R.A., MARTINS, J.L., TEIXEIRA, E.H., ALVES, F.C.S., RAMOS, V.S.C., SOUSA, G.S., CAVADA, B.S. & SANTOS, R.P. 2011. Compositional analysis of cashew (*Anacardium occidentale* L.) peduncle bagasse ash and its in vitro antifungal activity against *Fusarium* species. *R. bras. Bioci., 9*(2): 200-205.

SILVA, M.S.H. 2002. *Acajumembrana no cuidado cultural em feridas*. 146 p. Tese de doutorado, Universidade Federal do Ceará, Fortaleza, 2002.

STEPANOVIC, S., VUKOVIC, D., DAKIC, I., SAVIC, B. & SVABIC-VLAHOVIC, M. 2000. A modified microtiterplate test for quantification of staphylococcal biofilm formation. *J. Microbiol. Methods, 40*: 175-179.

TAKAHASHI, N & NYVAD, B. 2011. The role of bacteria in the caries process: ecological perspectives. *J. Dent. Res., 90*(3): 294-303.

TRIOLA, M. F. (2008) *Elementary Statistics*. 11th ed. England: Addison-Wesley Longman. p. 896.