Antimicrobial Activity of Cashew Nut Liquid: Study of the Microbial Resistance

Rodrigues, R.C.E.[1]; Silva, G.F.[2]; Albuquerque, P.M.[1]

[1]Programa Multicêntrico da SBBq, Escola Superior de Tecnologia, EST-UEA**,**
[2]PPG em Química, Dep. de Química, UFAM, Manaus-AM, Brazil.

INTRODUCTION. Microbial resistance represents a worldwide concern and the widespread use of antibiotics has aggravated this situation. Therefore, it is necessary to look for new sources of antimicrobial compounds. Anacardic acid (AA), present in cashew nut liquid (CNL), has demonstrated antimicrobial activity, but its action mechanism has not been explained. **OBJECTIVES:** Evaluate the antimicrobial activity of CNL and of the isolated AA on pathogenic microorganisms, and verify the presence of resistance structures. **MATERIAL AND METHODS:** Cashew nuts were collected in Coari-AM, and used for the extraction of CNL using hexane. The AA was then isolated from the CNL. The antimicrobial activity was evaluated through agar diffusion method at 4 mg/mL. MIC was determined by the microdilution method and MBC was verified in solid media. The beta-lactamase production in bacteria was investigated through the ceftazidime disc method. **RESULTS AND DISCUSSION:** A 26% yield on CNL extraction was obtained, and a 63% yield of AA. *S. aureus* and *S. pyogenes* were susceptible to both CNL and AA, while *E. coli*, *P. aeruginosa* and the pathogenic fungi (*Candida albicans*, *Aspergillus brasiliensis, Colletotrichum guaranicola, Colletotrichum gloeosporioides*, *Mycosphaerella fijiensis, Sclerotium rolfsii*, *Rhizoctonia* sp. and *Corynespora* sp.) were resistant. The MIC values for *S. aureus* and *S. pyogenes* were 15.62 and 62.5 µg/mL, respectively. The MBC values for *S. aureus* and *S. pyogenes* were 31.25 and 125 µg/mL, respectively. There was no significant difference ($p<0.05$) between CNL and AA, concerning its antimicrobial activity. In the study of resistance structures, the production of beta-lactamase was not observed. *E. coli* and *P. aeruginosa* resistance could be associated to cell wall structure, constituted essentially by a lipopolysaccharyde that blocks hydrophobic oils from penetrating and avoids their excess on the cell membrane. **CONCLUSIONS:** CNL and AA showed to be active against gram-positive bacteria and inactive against gram-negative bacteria, and phytopathogenic fungi. Resistance structures of fungi should be further investigated.

Key-words: *Anacardium occidentale*, antibiotics, microbial resistance.
Acknowledgments: EST-UEA, CAPES and FAPEAM.

REFERÊNCIAS BIBLIOGRÁFICAS

ABRAAM, E. P.; CHAIN, E. An enzyme from bacteria able to destroy penicillin. **Nature**, v. 146, p. 837-837, 1940.

ALVES, C. G.; BRANDÃO, H. N.; DAVID, J. P.; LIMA, L. S. Avaliação da atividade antioxidante de flavonóides. **Diálogos e Ciência**, n.12, 2007.

BOUTTIER, S.; FOURNIAT, J.; GAROFALO, C.; GLEYE, C.; LAURENS, A.; HOCQUEMILLER, R. b-Lactamase inhibitors from Anacardium occidentale. **Pharmaceutical Biology**, v. 40, p. 231-234, 2002.

CALO, S.; BILLMYRE, R. B.; HEITMAN, J. Generators of phenotypic diversity in the evolution of pathogenic microorganisms. **PLoS Pathogens**, v. 9, e1003181, 2013.

CANILLAC, N.; MOUREY, A. Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. **Food Microbiology**, v. 18, p. 261-268. 2001.

CASTRO, R. D., LIMA, E. O. Atividade antifúngica *in vitro* do óleo essencial de *Eucalyptus globulus* L. sobre *Candida* spp. **Revista de Odontologia UNESP**, 39,179-184, 2010.

COUTINHO, H. D. M.; BEZERRA, D. A. C.; LÔBO, K.; BARBOSA, I. J. F. Atividade antimicrobiana de produtos naturais. **Conceitos,** 2004.

CHAVES, M. H.; CITÓ, A. M. das G. L.; LOPES, J. A. D.; COSTA, D. A.; OLIVEIRA, C. A. A.; COSTA, A. F.; ELEODORO, F. M.; JUNÍOR, B. Fenóis totais, atividade antioxidante e constituintes químicos de extratos de *Anacardium occidentale* L., Anacardiaceae. **Revista Brasileira de Farmacognosia**, v. 20, p. 106-112, 2010.

FENNER, R.; BETTI, A. H.; MENTZ, L. A.; RATES, S. M. K. Plantas utilizadas na medicina popular brasileira com potencial atividade antifúngica. **Revista Brasileira de Ciências Farmacêuticas**, v. 42, p. 369-394, 2006.

GARAU, J. Beta-lactamases: current situation and clinical importance. **Intensive Care Medicine**, v. 20, p. 95-99, 1994.

ENGELMEIER, D., HADACEK, F. Antifungal natural products: assays and applications in: RAI, M.; CARPINELLA, M. C. (Eds.), **Naturally Occurring Bioactive Compounds**. Elsevier: Amsterdam, pp.423-467, 2006.

HOF, H. Is there a serious risk of resistance development to azoles among fungi due to the widespread use and long-term application of azole antifungals in medicine? **Drug Resistance Updates**, v. 11, p. 25-31, 2008.

JOY, M.; JOHN, J.; SMITHA, K. P.; NAIR, R.V. Inhibitory effects of cashew (*Anacardium occidentale* L.) on polypathogenic fungi. **Allelopathy Journal**, v. 13, p. 47-56, 2004.

KATTIMUTTATHU, S.I.; VADI, K.S. Synthesis, structure, and properties of novel polyols from cardanol and developed polyurethanes. **Industrial & Engineering Chemistry Research,** v. 44, p. 4504-4512, 2005.

KLEMCHUCK, P. P. Antioxidants. **Úllmann's encyclopedia of industrial chemistry,** v. A3, 5ed., 1999.

KÖNÖNEM, E.; EEROLA, E.; FRANDSEN, E. V.; JALAVA, J.; MÄTTÖ, J.; SALMENLINNA, S.; JOUSIMIES-SOMER, H. Phylogenetic characterization and proposal of a new pigmented species to the genus *Prevotella*: *Prevotella pallens* sp. nov. **International Journal of Systematic Bacteriology**, v. 48, p. 47-51, 1998.

LIMA, C. A. A.; PASTORE, G. M.; LIMA, E. D. P. A. Estudo da atividade dos ácidos anacárdicos do óleo da castanha de caju (CNSL) dos clones de Cajueiro-anão-precoce CCP – 76 e CCP – 09 em cinco estágios e maturação sobre microorganismos da cavidade bucal. **Ciência e Tecnologia de Alimentos***,* v. 20, p.358-362, 2000.

MILLER, J. D.; YOUNG, J. C.; TRENHOLM, H. L. *Fusarium* toxins in field corn. I. Time course of fungal growth and production of deoxynivalenol and other mycotoxins. **Canadian Journal of Botany**, v. 61, p. 3080-3087, 1983.

MORAES, R. J. Q.; ALMEIDA, C. A. A.; DILKIN, P.; KOWALSKI; MURMANN, L.; MALLMANN, C. A. Dosagem de ergosterol como indicador de contaminação fúngica em milho armazenado. **Arquivos do Instituto Biológico**, v.70, p.483-489, 2003.

MOTA, S. G. R. **Ensaio in vitro e análise quimiométrica de inibidores da enzima lanosterol 14α- desmetilase de *Moniliophthora perniciosa***. Dissertação. Programa de Pós-graduação em Biotecnologia, Universidade Estadual de Feira de Santana, Feira de Santana, 2009.

MOUCHREK FILHO, 2002
NASCIMENTO, J. S. **Biologia de Microrganismos**. Editora Universitária UFPB: João Pessoa, 2010.

NCCLS (NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS). **Padronização dos testes de sensibilidade a antimicrobianos**

por disco-difusão: approved standard. 8ed., v. 23 n.1, 2003 (atual CLSI - www.clsi.org).

NCCLS (NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS). **Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically**, approved standard. 15 ed., 2000.

NCCLS (NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS). **Método de referência para testes de diluição em caldo para determinação da sensibilidade a terapia antifúngica dos fungos filamentosos**, norma aprovada. 2002. (atual CLSI - www.clsi.org).

NCCLS (NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS). **Método de Referência para Testes de Diluição em Caldo para Determinação da Sensibilidade de Leveduras à Terapia Antifúngica:** Norma Aprovada. 2ed. M27-A2, v. 22, n. 15, 2002. (atual CLSI - www.clsi.org).

NCCLS (NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS). **Metodologia dos testes de sensibilidade a agentes antimicrobianos por diluição para bactéria de crescimento aeróbico**: Norma Aprovada. 6ed. M7-A6, v. 23, n. 2, 2007. (atual CLSI - www.clsi.org).

NAEWBANIJ, M.; SEIB, P.A.; BURROUGHS, R. Determination of ergosterol using thin-layer chromatography and ultraviolet spectroscopy. **Cereal Chemistry,** v. 61, p.385- 388, 1984.

OLIVEIRA, F. N. S. **Sistema de produção para manejo do cajueiro comum e recuperação de pomares improdutivos**. Embrapa Agroindústria Tropical, Fortaleza, 2007.

PRABHAKARAN, K.; NARASYANAN, A.; PAVITRAN, C. Cardanol as a dispersant plasticizer for an alumina/toluene tape casting slip. **Journal of the European Ceramica Society**, v. 21, p. 2873-2878, 2001.

SELMECKI, A.; FORCHE, A.; BERMAN, J. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. **Science**. v. 313, p. 367-370, 2006.

SCHNÜRER, J. Distribution of fungal biomass among fine bran, coarse bran, and flour from wheat stored at four different moisture levels. **Cereal Chemistry**, v. 68, p.434-437, 1991.

SOARES, B. V. **Estudo fitoquímico e antifúngico de extratos de plantas contra *Microsporum canis* e *Candida* spp. isolados de Cães**. Dissertação. Programa de Pós-Graduação em Ciências Veterinárias. Universidade Estadual do Ceará, Fortaleza, 2008.

SOUSA JUNIOR, M. A. FERREIRA, E. S.; CONCEIÇÃO, G. C. **Betalactamases de Espectro Ampliado (ESBL):** um Importante mecanismo de resistência bacteriana e sua detecção no laboratório clínico. NewsLab, ed. 63, p. 152-174, 2004.

TAVARES, W. Resistência bacteriana. In: **Manual de antibióticos e quimioterápicos antiinfecciosos** 3ª ed. Atheneu: São Paulo, 2001.

WILLIAMS, J. D. β-lactamases and β-lactamase inhibitors. **International Journal of Antimicrobial Agents**, v. 12, p. 3-7, 1999.

YOUNG, J. C. Microwave–assisted extraction of the fungal metabolite ergosterol and total fatty acids. **Journal of Agriculture and Food Chemistry,** v. 43, p.2904-2910, 1995.