

Chemical and Biological Analysis of Peptides in Skin Secretion of Two Species of *Aparasphenodon* (Anura, Hylidae)

Monken, F.F.^{1,2}; Prudêncio, E.R.¹; Cavalcanti, L.R.I.¹; Riger, C.J.¹; Chaves, D.S.A.²

¹Laboratório de Estresse Oxidativo em Microrganismos (LEOM), Dep. de Química, ICE-UFRRJ, Seropédica, Brazil. ²Laboratório de Bioativos Naturais, Dep. de Ciências Farmacêuticas (LQBioN), ICBS-UFRRJ, Seropédica, Brazil.

INTRODUCTION AND OBJECTIVES: Anurans have a defense system based on the secretion of bioactive substances, which peptides are the main compounds. Their biological activity is due to their structural arrangement. This work aims to chemically characterize the peptides of *A. brunoi* and *A. arapapa* skin secretions by isolation and purification of these compounds, followed by structural identification and biological analysis. **MATERIALS AND METHODS:** Individuals of *A. brunoi* (n=5) and *A. arapapa* (n=3) were collected at Ilha da Marambaia (Mangaratiba-RJ) and Ilhéus (BA), respectively. The secretions were obtained by electrical stimulation, with approximate yield 4mg by individual. Thin Layer Chromatography technique was applied to the secretions, using eluent butanol:acetic acid:water (7:2:1) and, as chemical revealing, a solution of ninhydrin (Ethanol 10%). The extracts were submitted to HPLC (RP18 5µ 25x4,0mm, isocratic 70% acetonitrile/water 1% acetic acid, 2,0mL.min⁻¹, 205nm) and, after that, to Mass Spectrometry (ESI, positive ion, 100-1600m/z). Biological assays were conducted in order to determine the toxicity, in which we exposed the secretion for 2 and 24h to eukaryotic cells of *Saccharomyces cerevisiae*. **RESULTS AND CONCLUSIONS:** Aminic groups were found in ninhydrin analysis by the appearance of the violet color. Furthermore, by using Liquid Chromatography, we detected, in both species, two dominant peaks with the same retention time (0.88 and 1.44 min), indicating the presence of similar polarity in both secretions. The molecular ions detected by Mass Spectrometry confirmed the presence of peptides with proximal molecular masses (*A. brunoi* 1199m/z, *A. arapapa* 1215m/z). The cellular viability assays did not show toxicity. In conclusion, the cutaneous secretions of those species present similar chemical composition with peptides of very similar size and polarities, which could indicate that they are the same molecule. This hypothesis can be confirmed after isolation and sequencing in following steps of this study.

Acknowledgements: Centro de Aperfeiçoamento de Pessoal do Ensino Superior (CAPES)

Keywords: Amphibian, skin glands, bioactive compounds.