Identification of peptides from *Phyllomedusa burmesteri* skin secretomics by Nano LC-MS/MS

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Recent improvements in mass spectrometry combining nano-scale liquid chromatography with electrospray ionization tandem mass spectrometry (nano LC-MS/MS) provide new tools to characterize complex mixtures of bioactive peptides. The aim of this study was to detect as many as possible peptides from manually collected extracts of the secretions from the total skin and from the parotoids glands of an amphibian specimen. For this propose, a method for comprehensive analysis of *P. burmeisteri* peptidome was developed. First, the peptides in the skin and parotoid secretion were harvested by ultrafiltration with a 10 kDa molecular weight cutoff. Then, the filtrate samples were directly analyzed by nano LC-MS/MS without trypsin digests. The MS/MS spectra were analyzed by *de novo* peptide sequencing and by MS/MS ion search Mascot 2.2 server. We analyzed 396 peptides in the total skin sample and 179 peptides in the parotoid sample. The MS/MS ion search led to the preliminary identification of 41 peptides from only one specimen of *Phyllomedusa*. These peptides were relatively short, many with post-translational modifications (pyroglutamic, and C-terminal amidation), and some belonged to antimicrobial peptides precursors. The filtrates of the secretions samples were also fractioned by RP - HPLC, and analyzed by MALDI-ToF MS. These analysis showed 75 and 43 *m/z* signals respectively to total skin and parotoid secretions, and some of these fractions have antimicrobial activity, that corroborates our previous findings. These results indicate the usefulness of this approach that, combined to specific biological assays, was able to successfully identify low-abundance bioactive peptides from a complex samples such this amphibian secretion.

KEYWORDS: Peptides, *Phyllomedusa burmeisteri*; mass spectrometry.

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