

## Characterization of the Skin Secretion of *Phyllomedusa tetraploidea*

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Substances found in most amphibians skin include bioactive molecules with high toxicity and less toxic components involved in the animal defense. This study aimed the comprehension of the action properties of the skin secretion of *Phyllomedusa tetraploidea*. The skin secretion was obtained by the animals immersion in alcoholic solution (5 % ethanol). These solutions were lyophilised and stored at -20 °C until use. The secretion showed an insoluble fraction corresponding to 50% of the sample in aqueous solution, though chemical assays were performed only with the soluble part, except by the zimogram analysis. Proteins concentration was performed by the method of Lowry. Proteolytic activity was determined by colorimetric assay, using azocasein as substrate and also by zimogram using polyacrilamide gel with gelatin (0,6 mg/ml). The hemolytic activity was assessed by the determination of hemoglobin level in the supernatant after exposal of human erythrocytes to the skin secretion. Toxicity was determined through intraperitoneal injection into male mice and the inflammatory cells migration counted 4h after intraperitoneal injection. The proteolytic activity results showed activity only in the insoluble fraction, as revealed by the zimogram and was abolished by EDTA, suggesting the presence of metalloproteinases. The electrophoretic profile showed high molecular weight proteins and a significant accumulation of peptides with mass below 14 kDa. The skin secretion showed high hemolytic activity in a dose-dependent manner, from 0.06 to 8 µg/ml but low toxicity, as DL50 could not be determined with doses up to 16 mg/kg. The analysis of the inflammatory infiltrate (4 to 8 mg/kg) activated the migration of polymorphonuclear leukocytes to the peritoneal cavity. These results present the importance of future studies of the toxins for pharmacological applications and as mediators of the immune system.

Keywords: amphibian, Phyllomedusa tetraploidea, skin secretion.

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