

Comparison of Antimicrobial and Cytotoxicity Profiles of Skin Secretions from Endemic Polypedates Cruciger and Cosmopolitan Duttaphrynus Melanostictus

K.W.L.R.T. Kumari^{1, 2*}, R. N. Jinadasa², R.S. Rajakaruna¹

¹*Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka*

²*Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka*

**lakshanikulathunga1204@gmail.com*

Antimicrobial peptides (AMPs) in amphibian skin secretions are being explored as potential new antibiotics. Cytotoxicity to mammalian cells is a major challenge in their clinical usage. This study explored antimicrobial properties of skin secretions from endemic *Polypedates cruciger* (common hourglass treefrog) compared to cosmopolitan *Duttaphrynus melanostictus* (Asian common toad) with known AMPs. Secretions were obtained from specimens collected at University of Peradeniya premises by electrical stimulation (5V, DC) and washing skin with deionized water. Secretions were stabilized with glacial acetic acid (50:1 v/v) and sterile filtered. Activity against *Staphylococcus aureus*, multidrug resistant (MDR) *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were determined using microdilution in 96-well plates with two-fold serial dilutions at 37⁰ C for 18-24 hours. Absorbance was measured at 620 nm, and viability was verified by growth on blood agar. Cytotoxicity was determined by incubating secretions with canine red blood cells (RBCs) at 37⁰ C with 0.1% Tween 20 as positive control. Kill curve assays were conducted against *S. aureus* and *E. coli* with undiluted skin secretions at 37⁰ C. All experiments were duplicated. *S. aureus* and *E. coli* were inhibited by *P. cruciger* and *D. melanostictus* secretions in 1:4 and 1:8 dilutions respectively. Both secretions also inhibited MDR *A. baumannii*, and *P. aeruginosa* at 1:4 dilution, indicating broad-spectrum activity. However, both secretions were inefficient against *K. pneumoniae*. *E. coli* was rapidly killed by *P. cruciger* secretions (10 min post exposure), while *D. melanostictus* secretions needed 1 hr exposure. Both secretions needed at least 2 hr exposure to kill *S. aureus*. Canine RBCs were not affected by *P. cruciger* secretions after 1 hr exposure, while ~20% haemolysis was caused by *D. melanostictus* secretions suggesting significantly lower cytotoxicity in *P. cruciger* secretion, indicating potential utility of AMPs from *P. cruciger* with reduced cytotoxicity as an antibiotic candidate.

Keywords: Antimicrobial Peptides, *Polypedates Cruciger*, *Duttaphrynus Melanostictus*, Cytotoxicity, *Acinetobacter Baumannii*, *Staphylococcus Aureus*