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Antibacterial activity of venom from *Philodryas nattereri* Steindachner, 1870[1]

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Resumo

Os venenos de serpentes contêm substâncias biologicamente ativas que apresentam uma gama de efeitos biológicos, tais como neurotóxicos, miotóxicos, antiparasitários e atividade antibacteriana. Este estudo teve como objetivo avaliar a atividade antimicrobiana do veneno de *Philodryas nattereri* sobre bactérias Gram-negativas e Gram-positivas. Esta atividade foi investigada contra cepas de *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella choleraesuis spp. choleraesuis* (ATCC 10708), utilizando o método de microdiluição em caldo. O veneno de *P. nattereri* inibiu significativamente o crescimento das cepas de *S. aureus*, *S. choleraesuis spp. choleraesuis* e, apresentou menor efeito em cepas de *P. aeruginosa*. O veneno não apresentou atividade frente a cepas de *E. coli*. Esses resultados demonstram o potencial da peçonha de *P. nattereri* como fonte de compostos antibacterianos.

Palavras-Chave: agente antibacteriano, inibição do crescimento, *Philodryas nattereri*, peçonha.

Antibacterial activity of venom from *Philodryas nattereri* Steindachner, 1870. Snake venoms are composed of active substances that present a diversity of biological effects such as neurotoxicity, myotoxicity, antiparasitic and antibacterial activities. This study was undertaken to evaluate the antimicrobial activity on Gram-negative and Gram-positive bacteria of the venom from *Philodryas nattereri*. This activity was investigated against *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), and *Salmonella choleraesuis* spp. *choleraesuis* (ATCC 10708) using a broth microdilution method. *Philodryas nattereri* venom significantly inhibited the growth of *Staphylococcus aureus*, *Salmonella choleraesuis* spp. *choleraesuis* and, to a lesser extent, *Pseudomonas aeruginosa*, but was inactive against *Escherichia coli*. These results indicate the potential of *P. nattereri* venom as a source of antibacterial compounds.

Key-words: antibacterial agent, growth inhibition, *Philodryas nattereri*, snake venom.

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1. Introduction

Snake venoms are composed of active substances that exert a variety of biological effects such as neurotoxicity, myotoxicity, cardiotoxicity, antiparasitic and antibacterial activities (GOMES et al., 2009; OLIVEIRA et al., 2010).

Protein and peptides make up 90-95% of the dry weight of venom. Some snake venoms also contain carbohydrate, lipid, biogenic amines, free amino acids and inorganic cations such as sodium, calcium, potassium, magnesium and small amounts of zinc, nickel, cobalt, iron, manganese (GUTIERREZ, 2002; SANTOS et al., 2008).

Snake venoms exert antimicrobial activity that may have developed as a defense mechanism against the microorganisms present in the prey (TALAN et al., 1991; SHIVIK, 2006).

Among some of the common antimicrobial components that have been isolated from snake venoms are the enzymes L-amino oxidase (LAAO) and phospholipase A_2 (PLA_2), such as LAAO purified of *Pseudechis australis* showed significant antimicrobial activity against different strains of *Aeromonas sp.* (STILES et al., 1991).

The study aimed to evaluate antibacterial activity of the venom from *P. nattereri* in different concentrations against *Staphylococcus aureus* (Gram-positive species), and three Gram negative bacteria, *Salmonella choleraesuis* spp. *choleraesuis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

2. Material and Methods

Snakes of *P. nattereri* were captured on Aroeiras Farm in the municipality of Upanema (5°38'32" S and 37°15'27"W), state of Rio Grande do Norte, Brazil, and transported to NUROF (Regional Nucleus of Ophiology). Venom pools were made from more than 40 individual snakes and collected from the venom gland into capillary tubes to prevent contamination with saliva. After the outflow of the venom into the capillary tube, the venom was frozen and lyophilized.

The bacterial strains used were *E. coli* (ATCC10536), *P. aeruginosa* (ATCC9027), *S.* spp. *choleraesuis* (ATCC10708) and *S. aureus* (ATCC6538). The antimicrobial activity was determined using a broth microdilution method as reported by Hecht et al. (2003), with modifications. Several concentrations of venom (0, 19 to 200µg/mL) were used.

The microbial strains were subcultured, and microbial density was adjusted as previously described (NERY et al., 2014). The cultures were diluted to 1:100 (~ 1.5 x 10^6 CFU/mL). An inoculum of 80 µL of microbial culture was added to 20 µL of each concentration of venom in BHI broth (100 µL) in 96-well plates. For the negative control, wells contained sterile PBS buffer (pH 7.4) instead of venom. For the positive controls (growth inhibition), wells contained culture medium, an antimicrobial agent (amikacin for bacteria and ketoconazole for yeast), and an inoculum of microorganisms. The plates were incubated at 35°C for 24 hours, and inhibition of microorganism growth was determined based on the turbidity measured at 490 nm.

The antimicrobial potency was evaluated based on the lowest venom concentration required to inhibit microbial growth (detected by a lack of visible turbidity). The results were expressed as means ± SEM (n = 6). Statistical evaluation was determined by analysis of variance (ANOVA) followed by the Bonferrni test. Statistical significance was set at 5%. The programs used to perform the statistical analysis were Microsoft Excel 2007 and Prism 5.0 (GraphPad Inc., La Jolla, CA, USA).

3. Results and Discussion

Philodryas nattereri venom significantly inhibited the growth of gram-positive *S. aureus* at concentrations of 0.391, 0.782, 3.125, 6.25, 12.5 and 25µg/mL. The growth of Gram-negative *P. aeruginosa* and *S. choleraesuis* spp. *choleraesuis* was inhibited by *P. nattereri* venom at concentrations of 1.563, 25 and 200µg/mL and 0.782, 6.25, 12.5, 25, 100 and 200µg/mL, respectively, whereas the growth of *E. coli* was not attenuated at any venom concentration (Figure 1).

Previous studies have demonstrated the antimicrobial effects of snake venoms and isolated substances. In the present study, the venom from *P. nattereri* demonstrated a significant antibacterial effect against *S. aureus*, *P. aeruginosa* and *S. choleraesuis* spp. *choleraesuis*, while in strain of *E. coli* was no observed antibacterial effect in all concentrations of venom.

The evaluation of antimicrobial effect of venom snakes was determined in different

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species, such as *Calloselasma rhodostoma* and *Ophiophagus hannah* in strains of *S. aureus*, and *Bothrops marajoensis* venom in strains of *P. aeruginosa* and *S. aureus* (SAN et al., 2010; TORRES et al., 2010).

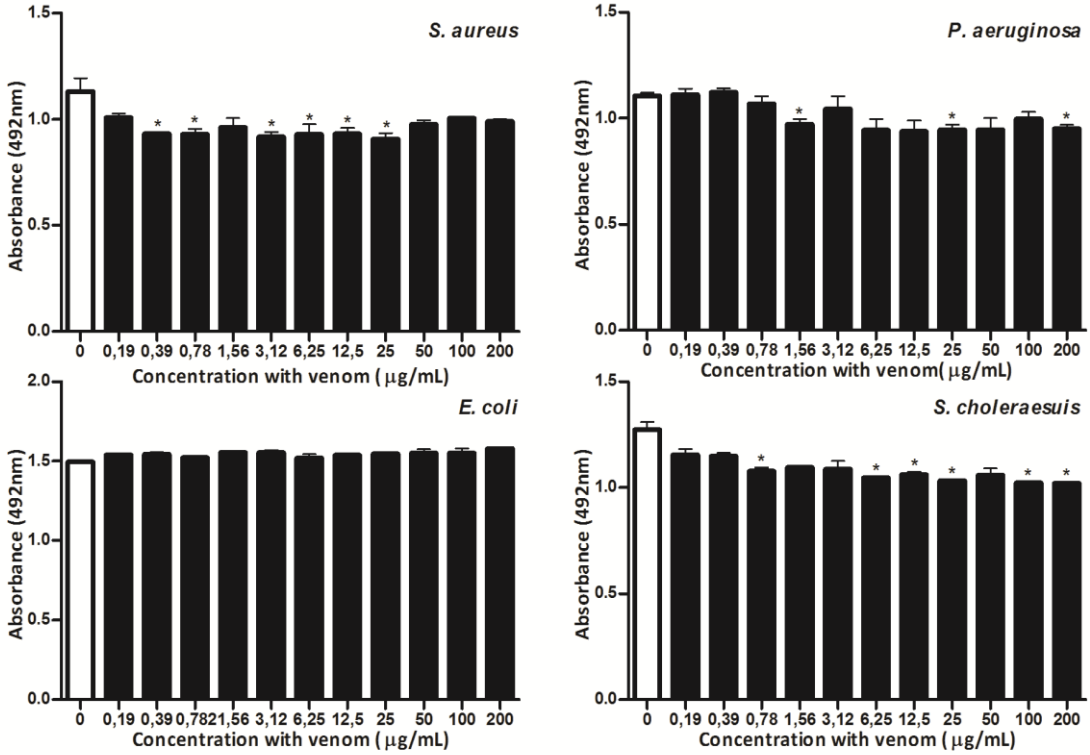

Figure 1 - Inhibition of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella choleraesuis* spp. *choleraesuis* by *P. nattereri* venom (0.19-200µg/mL). Bacterial growth was monitored spectrophotometrically (492 nm). The values represent the mean ± SEM (n = 6). *p < 0.05 when compared with the corresponding control group.

The antimicrobial activity in snake venoms was due to enzymatic components, such as phospholipase A_2 (PLA_2) and L-amino oxidase (LAAO), which suggests the importance of the subproduct of the enzymatic action of LAAO is hydrogen peroxide and ammonia, because the antibacterial effects of LAAOs from snake venom are significantly diminished by the actions of catalase (Wei et al. 2003; Toyama et al., 2006; Samy et al., 2007). In this study was observed resistance of *E. coli* against venom from *P. nattereri*, probably the mechanism of resistance is possible due the outer membrane of gram-negative bacteria (DEVINE and HANCOCK, 2002).

4. Conclusions

In summary, we have successfully established the potential of antimicrobial activity using snake venom from *P. nattereri* against strains of *S. aureus*, *P. aeruginosa* and *Salmonella choleraesuis* spp. *choleraesuis*, but no observed for strain of *E. coli*. Further studies will be required in order to elucidate the mechanisms involved in this toxicity.

Disclosure

This article is unpublished and not being considered for any other publication. The author(s) and reviewers did not report any conflict of interest during their evaluation. Therefore, the Journal Scientia Amazonia owns the copyright and has the approval and permission of authors for publication this article electronically.

References

DEVINE, D. A., HANCOCK, R. E. W. HANCOCK. Cationic peptides: Distribution and mechanisms of resistance. **Current Pharmaceutical Design**, v. 8, p. 703-771, 2002.

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GOMES, M. S. R., MENDES, M. M., OLIVEIRA, F., ANDRADE, R. M., BERNARDES, C. P., HAMACUCHI, A. BthMP: a new weakly hemorrhagic metalloproteinase from *Bothrops moojeni* snake venom. **Toxicon**, v. 53, p. 24-32, 2009.

GUTIÉRREZ, J. M. Comprendiendo los venenos de serpientes: 50 años de investigaciones en América Latina. **Revista de Biologia Tropical**, v. 50, p. 377-394, 2002.

HECHT, D.W., CITRON, D.M., COX, M., JACOBUS, N., JENKINS, S. G., ONDERDONK, A. **Methods for dilution in antimicrobial susceptibility tests for bacteria that grow aerobically**. National Committee for Clinical Laboratory Standards, 2003.

NERY, M. D. A., ALVES, N. T. Q., ALVES, R. S., SOUSA, D. F., MENEZES, D. B., NERY, E. A., AQUINO, H. D., RIBEIRO, R. T. M., MONTEIRO, H. S. A. The renal effects and initial characterization of venom from *Philodryas nattereri* Steindachner, 1870. **Toxicology reports**, v. 1, p. 812-819, 2014.

OLIVEIRA, F. N., BRITO, M. T., MORAIS, I. C. O., FOOK, S. M. L., ALBUQUERQUE, H. N. Accidents caused by *Bothrops* and *Bothropoides* in the State of Paraiba: epidemiological and clinical aspects. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 43, p. 1-6, 2010.

SAMY, R. P., GOPALAKRISHNAKONE, P., THWIN, M. M., CHOW, T. K. V., BOW, H., YAP, E. H., THONG, T. W. J. Antibacterial activity of snake, scorpion and bee venoms: A comparison with purified venom phosholipase A_2 enzymes. **Journal of Applied Microbiology**, v. 102, p. 150-159, 2007.

SAN, T. M., VEJAYAN, J., SHANMUGAN, K., IBRAHIM, H. IBRAHIM. Screening antimicrobial activity of venoms from snakes commonly found in Malaysia. **Journal of Applied Sciences**, v. 10, p. 2328-2332, 2010.

SANTOS, M. M. D. V., SANTANA, C. D., GIGLIO, J. R., SILVA, R. J., SAMPAIO, S. V., SOARES, A. M. Antitumoural effect of an *L*-amino acid oxidase isolated from *Bothrops jararaca* snake venom. **Basic and Clinical Pharmacology and Toxicology**, v. 102, p. 533-542, 2008.

SHIVIK, J. A. Are vultures birds and do snakes have venom, because of macro and microscavenger conflict? **BioScience**, v. 56, p. 819-823, 2006.

STILES, B. G., SEXTON, F. W., WEINSTEIN, S. A. Antibacterial effects of different snake venoms: Purification and characterization of antibacterial proteins from *Pseudechis australis* (Australian King Brown or mulga snake) venom. **Toxicon**, v. 29, p. 1129-114, 1991.

TALAN, D. A., CITRON, D. M., OVERTURF, G. D., SINGER, B., FROMAN, P., GOLDSTEIN, E. J. Antibacterial activity of crotalid venoms against oral snake flora and other clinical bacteria. **The Journal of Infectious Diseases**, v. 164, p. 195-198, 1991.

TORRES, A. F. C., DANTAS, R. T., TOYAMA, M. H., FILHO, E. D., ZARA, F. J., QUEIROZ, M. G. R. Antibacterial and antiparasitic effects of *Bothrops marajoensis* venom and its fractions: Phospholipase A_2 and L-amino acid oxidase. **Toxicon**, v. 55, p. 795-804, 2010.

TOYAMA, M. H., TOYAMA, D. O., PASSERO, L. F. D., LAURENTI, M. D. L., CORBETT, C. E., TOMOKANE, T. Y. ET AL. Isolation of a new L-amino acid oxidase from *Crotalus durissus cascavella* venom. **Toxicon**, v. 47, p. 47-57, 2006.

WEI, J. F., WEI, Q., LU, Q. M., TAI, H., JIN, Y., WANG, W. Y., XIONG, Y. L. Purification, characterization and biological activity of ·-amino acid oxidase from *Trimeresurus mucrosquamatus* venom. **Acta Biochimica et Biophysica Sinica**, v. 35, p. 219-224, 2002.