

## POTENTIAL OF *Clusia fluminensis* FRUIT EXTRACTS TO NEUTRALIZE STAGES OF SNAKE POISONING

Pietroluongo, M.<sup>1,2\*</sup>; Fuly A. L.<sup>3</sup>; Da Silva A. R.<sup>3</sup>; Valverde, A. L.<sup>4</sup>; Paiva, S. R.<sup>1,5</sup>;

<sup>1</sup>Pós-Graduação em Ciências Aplicadas a Produtos para a Saúde, Faculdade de Farmácia, Universidade Federal Fluminense, RJ, Brazil

<sup>2</sup>FarManguinhos, Fundação Oswaldo Cruz, RJ, Brazil

<sup>3</sup>Departamento de Biologia Celular e Molecular, Universidade Federal Fluminense, RJ, Brazil

<sup>4</sup>Departamento de Química Orgânica, Universidade Federal Fluminense, RJ, Brazil

<sup>5</sup>Departamento de Biologia Geral, Universidade Federal Fluminense, RJ, Brazil;

[\\*mpietroluongo@gmail.com](mailto:mpietroluongo@gmail.com)

### Introduction

*Clusia fluminensis* is a native and endemic plant in Brazil, chemically characterized by the presence of xanthenes, benzophenones, flavonoids, coumarins, terpenoids, steroids and other substances with important biological activity. Previous studies have shown that the species has the potential to neutralize some local effects caused by snakebite venom (DA SILVA et al, 2019). Snake venom is a complex mixture capable of interfering with the central nervous, cardiovascular, muscular, hemostatic, renal and vascular systems (MATSUI et al., 2000). Ophidian accidents are classified by the World Health Organization as a neglected tropical disease, with socio-economic impact in the country due to deaths or irreversible sequelae that prevent family support activities. The lethality rate is low, but the consequences left by these accidents (paralysis and limb amputation) constitute a serious public health problem. Treatment with anti-ophitic serum is quite effective in reversing the systemic effects caused by the venom on the victim's body, but ineffective in neutralizing local effects associated with morbidities, on the affected region of the body (DA SILVA et al., 2007). In this context, the search for substances of plant origin has been shown to be relevant, particularly when directed to complementary therapies. The objective of this study was to evaluate the potential of *Clusia fluminensis* to inhibit the effects caused by the *Bothrops jararaca* snakebite, aiming to contribute to the production of therapeutic alternatives to support sorotherapy.

### Method

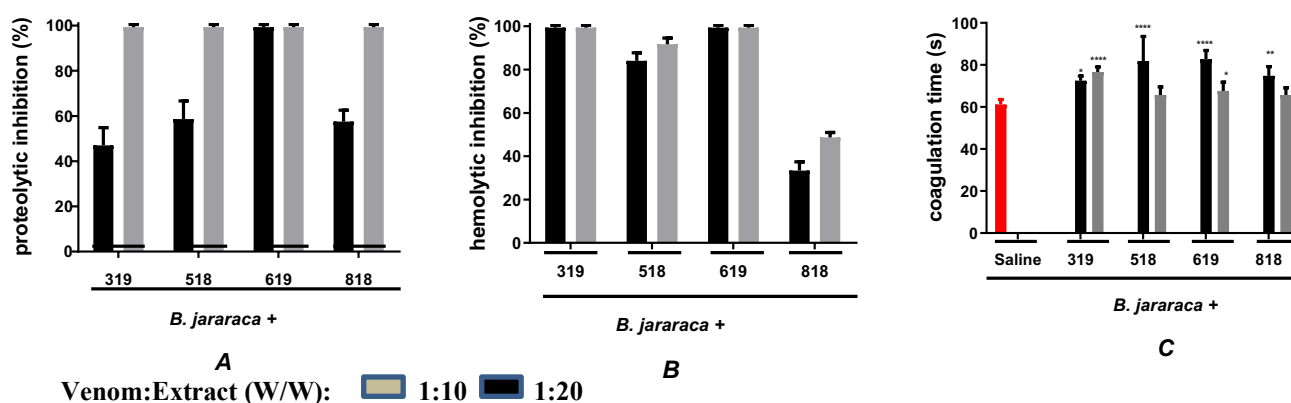
The assays were conducted with aqueous extracts of *C.fluminensis* fruits and *Bothrops jararaca* venom. The aqueous extracts were prepared by infusion as recommended by the phytotherapy formulary of Brazilian pharmacopoeia, in a proportion of 1: 8 (phytotherapeutic drug: water) and subsequently lyophilized. Extracts of *C. fluminensis* fruits collected in May and August of 2018 (samples 518 and 818) and March and June of 2019 (samples 319 and 619) were used. The samples were subjected to tests for proteolytic, hemolytic, and coagulation activities. Proteolytic activity: the neutralizing effect of the fruit extract was evaluated by pre-incubating the venom: extract (1:10 and 1:20) for 30 minutes at room temperature. Aliquots of the venom were incubated with 0.2% (w/v) azocasein. The samples were incubated at 37° C and after 90 minutes the reaction was stopped by adding of 0.4 mL of 10% trichloroacetic acid. The tubes were centrifuged, and 1 mL of the supernatant was added to 0.5 mL of 2N NaOH. Proteolytic activity was quantified in a spectrophotometer, 420 nm. Hemolytic activity: the degree of hemolysis was determined by an indirect hemolytic test with human red cells and chicken egg yolk emulsion as a substrate. *Bothrops jararaca* venom was incubated with the extracts (1:10 and 1:20 w / w, venom: extract) for 30 minutes at 25° C. Control experiments were carried out by incubating the venoms with saline solution without extracts or adding only the extracts to the reaction medium without the venom. Coagulation activity:

different concentrations of the venom were added to the plasma and the coagulation time was monitored for 60 seconds. *B. jararaca* venom was incubated for 30 min at 25° C with the extract from the fruit of *C. fluminensis* 1:10 and 1:20 w/w (venom: extract), the mixture was added to the plasma and coagulation was monitored.

## Results / Discussion

*B. jararaca* venom was incubated with the *C. fluminensis* fruits extracts encoded as 319, 518, 619 and 818. The extracts inhibited the proteolytic (Figure 1A), hemorrhagic (Figure 1B) and coagulant (Figure 1C) activities of the *B. jararaca* venom, demonstrating action on enzymes involved in the envenomation.

**Figure 1:** *In vitro* effects of *Clusia fluminensis* fruit extracts on *Bothrops jararaca* venom.



The metalloproteinases and serine proteases enzymes act in the envenomation pathogenesis with tissue damage, inflammation and disorders in the hemostatic system. The polyisoprenylated benzophenones and triterpenes, identified in preliminary chemical prospecting of *C. fluminensis* fruit extracts, can act on these enzymes and the results found suggest its ability to neutralize some local toxic effects involved in envenomation (MARTINS et al, 2009).

## Conclusion

The results obtained in this work demonstrated the efficacy of the aqueous extracts of *C. fluminensis* in neutralizing the venomous activities of *B. jararaca* venom. This species shows therapeutic potential in cases of snakebite poisoning, stimulating further studies such as the evaluation of metabolic production.

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