



## Inhibitory action of hesperetin on a venom metalloprotease from the *Bothrops asper* snake.

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### Abstract

A large portion of ophidian accidents in South America are provoked by snakes of the genera *Bothrops* and *Crotalus*, which have proteases that are zinc(II) dependent. Little is known in regard of flavonoids as inhibitors of metalloproteases, nevertheless, these secondary plant metabolites, such as hesperetin, can effectively bind with zinc(II). After obtaining the pure enzyme (BaP1) and tests that demonstrated the chelating effect of hesperetin with zinc(II) ions, we can propose that hesperetin can act as a possible inhibitor for metalloproteases that are zinc(II) dependent.

### Key words:

Metalloprotease, Snake venom, Hesperetin

### Introduction

In Latin America, a large proportion of ophidian accidents is due to snakes of the *Viperidae* family, more specifically the genera *Bothrops* and *Crotalus*, which have zinc(II)-dependent metalloproteases in their venoms, among other enzymes and compounds.

Hesperetin is a bioflavonoid belonging to the class of flavonones. There are studies that report on the chelating effect of flavonoids on metal ions and inhibitory action on enzymes, as recently tested by the use of hesperetin and naringin as inhibitors of Chikungunya virus replication.

With this in mind, this research project aimed to understand the inhibitory potential of hesperetin with the BaP1 metalloprotease, present in the venom of the *Bothrops asper* (*B. asper*) snake.

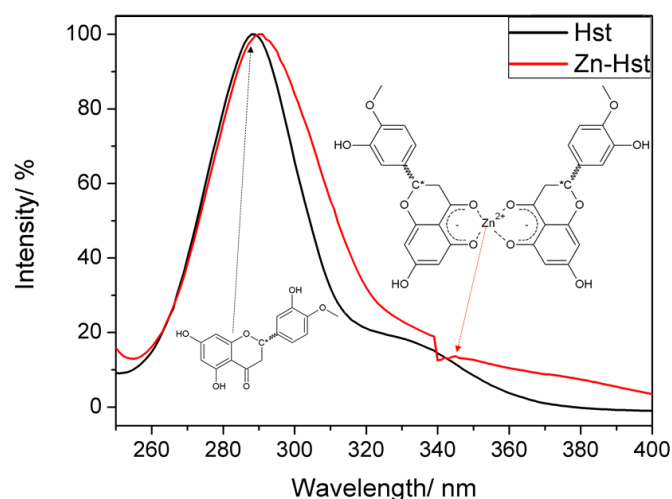
### Results and Discussion

Purification of the *B. asper* venom proteins included two liquid chromatography steps, one gel filtration (FPLC-DEAD) and the other by affinity (RP-HPLC), resulting in a pure protein named BaP1.

The purity of the protein was verified by gel electrophoresis, showing that BaP1 has a molar mass of around 22.8 kDa. Activity of this protein was verified using azocasein, a substrate which upon cleaving releases the azo group, a chromophore which can be analysed by UV-Vis at 595 nm.

Biophysical analyses were initiated, and spectra of circular dichroism and fluorescence were indicative for folded proteins.

Results obtained by UV-Vis show that there is a displacement of the absorbance of hesperetin (Hst) when mixed with a saturated solution of  $ZnCl_2$  in organic medium (Figure 1), which may indicate that the hydroxyl groups at positions 5 and 7 of ring A of hesperetin chelate with the  $Zn^{2+}$  ions, contributing to the idea that it would be possible to delay the action of the metalloprotease by this Hst chelating effect.



**Image 1.** UV-Vis spectra of free hesperetin ( $0,07 \text{ mg/cm}^{-3}$  of ethanol) and hesperetin with the saturated solution of  $ZnCl_2$  in ethanol (Hst  $0,07 \text{ mg/cm}^{-3}$  of  $ZnCl_2$  solution). A shift in maxima from 288 nm to 290 nm and spectral pattern breakage at 345 nm were observed.

### Conclusions

With the present study and with the results of the hesperetin complexation test with the  $ZnCl_2$  solution, it is possible to state that hesperetin has shown chelator properties in regard to  $Zn^{2+}$  ion, important for the BaP1 activity.

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