Antivenom properties of *Pentalinon* sp. (Apocynaceae) against the venom of *Bothrops asper*

Pérez-Bautista, Ivonne[1]; Neri-Castro, Edgar E.[2]; Alagón, Alejandro[2]; Zarzosa, Vanessa[2]; Cadena-Iñiguez, Jorge[3]; Soto-Hernández, Marcos[4]; Bravo-Vinaja, María Guadalupe[5]; Aguiñiga-Sánchez, Itzen[7]; Ramírez-Ramírez, Iván[7]; Ruiz-Posadas, Lucero del M.[1*]

[1] Colegio de Postgraduados, Campus Montecillo, Programa de Fisiología Vegetal, Texcoco, Estado de México, México. C.P. 56264.
[2] Universidad Nacional Autónoma de México, Instituto de Biotecnología, Col. Chamilpa, Cuernavaca, Morelos. C.P. 62210.
[3] Colegio de Postgraduados, Campus San Luis Potosí, Postgrado Innovación en Manejo de Recursos Naturales, Iturbide 73, Salinas de Hidalgo, San Luis Potosí, Mexico. C.P. 78600
[4] Colegio de Postgraduados, Campus Montecillo, Programa de Botánica, Texcoco, Estado de México, México. C.P. 56264.
[5] Colegio de Postgraduados, Campus Montecillo, Programa de Ganadería, Texcoco, Estado de México, México. C.P. 56264.
[6] Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Zaragoza, Laboratorio de Hematopoyesis y Leucemia, Iztapalapa, Ciudad de México. C.P. 09230.
[7] Colegio de Postgraduados, Campus Montecillo, Programa de Genética, Texcoco, Estado de México, México. C.P. 56264.
* Correspondence: lucpo@colpos.mx

Citation: Pérez-Bautista, I., Neri-Castro, E. E., Alagón, A., Zarzosa, V., Cadena-Iñiguez, J., Soto-Hernández, M., Bravo-Vinaja, M. G., Aguiñiga-Sánchez, I., Ramírez-Ramírez, I., & Ruiz-Posadas, L. del M. (2025). Antivenom properties of *Pentalinon* sp. (Apocynaceae) against the venom of *Bothrops asper*. *Agro Productividad*. https://doi.org/10.32854/efc7fv39

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Juan Francisco Aguirre Medina

Received: August 5, 2025.
Accepted: November 24, 2025.
Published on-line: January XX, 2026.

Agro Productividad, 18(12). December. 2025. pp: 171-183.

ABSTRACT

Objective: To evaluate the antivenin activity of organic extracts obtained from roots, stems and leaves of *Pentalinon* sp.

Design/methodology/approach: The phytochemical profile of the extracts was determined, and their inhibitory capacity was assessed against several effects induced by *Bothrops asper* venom, including lethality, proteolytic activity on azocasein, phospholipase A_2 activity, and fibrinogenolytic activity.

Results: The extracts of *Pentalinon* sp. predominantly contained flavonoids and terpenoids. None of the tested extracts were able to neutralize the venom's lethality. However, the ethanolic leaf extract completely inhibited proteolytic activity on azocasein and partially reduced phospholipase A_2 activity.

Findings/conclusions: Root extracts partially inhibited proteolytic activity on azocasein and prevented the degradation of the alpha and beta chains of fibrinogen.

Keywords: Contrayerba, snakebite, traditional medicine, phytochemistry.

INTRODUCTION

Mexico is a country with great biodiversity, with around 4,000 species of medicinal plants (Ocegueda *et al.*, 2005) and 93 species of venomous snakes (Uetz *et al.*, 2024), which generally cause an average of 3,800 cases of bites (Neri-Castro *et al.*, 2020). The most affected population are children and agricultural workers living in rural areas, where medical services do not always have antivenom (Sevilla-Sánchez *et al.*, 2021). One of the snake species of medical importance that causes a high number of snakebite accidents in Mesoamerica and northern South America is *Bothrops asper*, known as the nauyaca, fournosed viper, or mahuaquite (Mora-Obando *et al.*, 2023). Its venom is mainly composed of three families of toxins: a) phospholipases A2 (PLA2) that are associated with effects such as edema, myotoxicity, cytotoxicity; b) snake venom metalloproteinases (SVMP) that act mainly in the microvasculature generating local and systemic hemorrhages, coagulopathies, myonecrosis, which affect hemostasis; and c) snake venom serine proteases (SVSP) that mainly contribute to defibrinogenation (Gutiérrez and Rucabado, 2000; Alape-Girón *et al.*, 2008; Gutiérrez *et al.*, 2009; Mora-Obando *et al.*, 2023).

Given the difficulties in obtaining antivenoms (Vásquez *et al.*, 2024), people often resort to using plant-based treatments to delay or counteract the effects of the venom; however, in most cases the plants used do not have scientific studies that validate their use (Bénard-Valle *et al.*, 2015), such is the case of contrayerba (*Pentalinon* sp.), used in traditional Mayan and Huastec medicine (Pulido and Serralta, 1993; Pérez-Bautista *et al.*, 2023).

In recent years, the search for inhibitors that complement antivenom therapy has gained importance (Gutiérrez *et al.*, 2021) and provide more time to reach the nearest medical service. In Mexico, there are few scientific studies evaluating the antivenom activity of plants that can help treat snakebites. Therefore, the objective was to evaluate the antivenom properties of *Pentalinon* sp. extracts against the venom of *Bothrops asper*.

MATERIALS AND METHODS

Location and collection of plant material

Roots, stems, and leaves of *Pentalinon* sp. were collected from wild populations in February and April of 2023 and 2024 in the municipality of San Felipe Orizatlán, Hidalgo, Mexico (21° 05' and 21° 24' N and 98° 27' and 98° 42' W), and in the municipality of San Martín Chalchicuautla, San Luis Potosí, Mexico (98°40'31.912" N and 21° 19' 28.950" W).

The experimental phase was carried out in the Phytochemistry Laboratory and the Laboratory of Biological Tests with Medicinal Plants at the Postgraduate College, Montecillo Campus, as well as in the Molecular Medicine and Bioprocesses Laboratory of the Institute of Biotechnology at the National Autonomous University of Mexico.

Taxonomic identification and extraction of *Pentalinon* sp.

The identification of *Pentalinon* sp. was carried out through molecular analysis at the Institute of Biology of the National Autonomous University of Mexico. Ethanolic extracts and extraction pools were obtained from dried roots, stems, and leaves (Table 1) by maceration with 70% ethanol, 96% ethanol, hexane, ethyl acetate, or methanol. Filtration

Table 1. Amount of plant material used to obtain organic extracts of *Pentalinon* sp.

Extract	Sample weight (g)	Abbreviation
Pentalinon sp. root ethanol	253.6	R
Pentalinon sp. stem ethanol	264.1	T
Pentalinon sp. leaf ethanol	87.7	H
Pentalinon sp. root pool	54	PR
Pentalinon sp. stem pool	150	PT
Pentalinon sp. leaf pool	128.1	PH

was performed every three days with solvent exchange until the absence of color was observed. The crude extract was obtained by removing the solvent with a rotary evaporator (IKA® RV10, automatic control/BUCHI R-114 Equipan S.A. de C.V., Switzerland). Extraction pools were formed from the mixture of the extracts obtained with hexane, ethyl acetate, and methanol. The extracts were kept refrigerated at 4 °C until use.

Obtaining the Phytochemical Profile of *Pentalinon* sp. extracts

The presence of phenols, flavonoids, tannins, saponins, and terpenes was evaluated using thin-layer chromatography (TLC) (Wagner and Bladt, 2009). 0.4 g of each extract were weighed and dissolved in 8 mL of methanol. Samples of each extract were placed on 10×10 cm aluminum plates (HPTLC Silica gel 60 F254 Merck). They were eluted using different elution systems according to the metabolite group. Finally, they were observed under ultraviolet light at 365 nm and developed with different developers (Table 2).

Bothrops asper venom

A pool of lyophilized *Bothrops asper* venom was used, obtained from 39 adult specimens. This venom came from the venom bank of the Biotechnology Institute of the National Autonomous University of Mexico (UNAM). The pool was stored at −20 °C until use.

Animal use

Mice (18-20 g) of the ICR strain, of mixed sex, were used. The mice were kept in acrylic boxes (30×15×15 cm) with a 12 h light/12 h dark cycle, with unlimited access to

Table 2. Elution system and developer used for each group of metabolites analyzed in *Pentalinon* sp.

Metabolite	Elution system (v/v)	Developer
Phenols	Ethyl acetate:methanol (9:1)	Folin-Ciocalteu Reagent
Flavonoids	Ethyl acetate:methanol:water:formic acid (50:2:3:6)	NP-PEG
Tannins	Ethyl acetate:methanol (1:1)	
Saponins	Ethyl acetate:methanol (1:1)	Ferric Chloride
Terpenoids	Toluene:chloroform:ethanol (4:4:1)	Vanillin and 1% Sulfuric Acid in Ethanol
Alkaloids	Methanol-dichloromethane (1:9)	Anisaldehyde-Sulfuric Acid

food and water (Laboratory Rodent Diet). Bioethical approvals were obtained from the Biotechnology Institute of UNAM, the project number is 495.

Median lethal dose (LD_{50}) of *B. asper* venom and lethality neutralization with *Pentalinon* sp. extracts. The median lethal dose (LD_{50}) of the venom was obtained according to the methodology of Lorke (1983) with modifications. Three mice were inoculated intraperitoneally (IP) per dose of venom resuspended in phosphate buffer (PBS). The doses administered were 60, 100, 130, 150, and 170 μg mouse^{-1}. Deaths were recorded 24 h post-inoculation.

The evaluation of the lethality-neutralizing activity was determined using the methodology of Otero *et al.* (2000), with some modifications. Three mice were inoculated IP per treatment. The treatments were based on $3LD_{50}$ of the venom, which was pre-incubated for 30 min at 37 °C with different doses of extract. Controls consisted of $3LD_{50}$ of venom and some doses of extract (Table 3). Mice were monitored for 24 h, and mortality was recorded.

Inhibition of the proteolytic effect on azocasein

The methodology of Saravia-Otten *et al.* (2021) was followed with modifications. A dose of 1:48 venom:extract (w/w) was selected for evaluation; venom (20.5 μg) was used as a positive control, PBS as a blank, and PBS:DMSO:Tween 20% (1:0.2:0.2 v:v) as a negative control. Extracts with and without substrate were also included. All assays had a final volume of 20 μL and were pre-incubated at 37 °C for 30 min. 100 μL of an azocasein solution (Sigma A2765) of 10 mg mL^{-1} in an azocasein buffer was added, and the mixture was incubated for 30 min at 37 °C. Trichloroacetic acid (5%) was added to precipitate undigested proteins and stop the reaction. In 96-well plates, 150 μL of 0.5 M NaOH and 150 μL of supernatant were added. Absorbance was quantified at 450 nm using a BioTek

Table 3. Treatments evaluated for the neutralization of $3LD_{50}$ of *B. asper* venom with *Pentalinon* sp. extracts inoculated into mice (ICR).

Treatment	Venom	Extract (mg/mouse)
Venom*	3 LD_{50}	
Venom + ethanolic root extract	3 LD_{50}	5 20
Venom + ethanolic leaf extract	3 LD_{50}	20
Venom + ethanolic stem extract	3 LD_{50}	20
Venom + pool of leaf extracts	3 LD_{50}	20 50 80
Ethanolic root extract*	-	5 20
Ethanolic extract of stems*	-	20
Ethanolic extract of leaves*	-	20
Pool of leaf extracts*	-	20
Pool of root extracts*	-	20

*: control.

Elx800 microplate reader with Gen5 Version 2.0 software. Experiments were performed in triplicate. The absorbance of the extracts without substrate was subtracted from the activity of the extract incubated with the substrate to calculate the percentage of activity. Results were expressed as a percentage of proteolytic activity, considering 100% of the proteolytic activity generated by the venom.

Inhibition of phospholipase A2 (PLA2) activity

The evaluation was performed according to the methodology of Gutiérrez *et al.* (1988) with modifications. A selected dose of 1:623 venom:extract (w:w) was evaluated. As positive controls, *B. asper* venom (3 μg) and *Micrurus tener* venom were used; as negative controls, extracts, PBS, and PBS:DMSO:Tween 20% (1:0.2:0.2 v:v) were used. The venom:extract mixtures were pre-incubated for 30 min at 37 °C. The treatments were plated in triplicate on 0.1% agarose plates containing 2.0% egg yolk and rhodamine 6G. Subsequently, they were incubated for 20 h at 37 °C. The diameter of the halos was measured using GIMP 2.10.38. The average diameter of the halo generated by *B. asper* venom was taken as 100% phospholipase A2 activity.

Inhibition of fibrinogenolytic activity

The methodology described by Ware *et al.* (1942) was followed with some modifications. Mixtures of 50 μg of human fibrinogen with 1:221 and 1:442 venom:extract (w:w) doses were incubated for 30 min at 37 °C. Fibrinogen, venom (10 μg) with fibrinogen, and extracts with fibrinogen were used as controls. After incubation, a 6 μL aliquot of the sample was taken and mixed with 3 μL of 5x sample buffer containing β-mercaptoethanol, bringing the final volume to 15 μL with distilled water. This mixture was boiled for 5 min and centrifuged at 13,000 rpm for 30 s. Perform electrophoresis under reducing conditions with 12.5% gels.

Statistical Analysis

The median lethal dose data were analyzed using GraphPad Prism Version 10.0 software, plotting the percentage of mortality as a function of the logarithm of the amount of venom using the sigmoid dose-response nonlinear regression method. The neutralization data were analyzed using GraphPad Prism Version 10.2.3 software. For the proteolytic inhibition and phospholipase A2 assays, a one-way ANOVA was performed to determine if there was statistical significance. Subsequently, a Dunnett's test was performed to determine if the treatments were significantly different from the control (venom). A p-value <0.05 was considered significant. The analyses and graphs were performed using GraphPad Prism Version 10.2.3 software.

RESULTS AND DISCUSSION

Taxonomic identification of *Pentalinon* sp.

According to the results of molecular analysis using the matK (maturase K) molecular marker for plant identification (Letsiou *et al.*, 2024), 100% pairwise identity and identical sites correspond to the genus *Pentalinon*. This genus has only two species, *Pentalinon*

andrieuxii and *Pentalinon luteum*. In this case, it is suggested that the species used is *Pentalinon andrieuxii*, based on evaluations of its morphological characteristics (results not shown) and the distribution of both species: *P. luteum* is reported mainly in Florida and the Caribbean islands, and *P. andrieuxii* in various states of Mexico and South America.

Obtaining extracts and phytochemical profile of *Pentalinon* sp.

Three ethanolic extracts and three extract pools were obtained; the yields are presented in Table 4. The pools were obtained by increasing the polarity of the extractions to achieve complete extraction of the metabolites.

Flavonoids, terpenoids, and saponins were found in greater quantities in the roots, stems, and leaves; phenols, tannins, and alkaloids were present in smaller quantities and were not found in the roots (Figure 1).

Some of the ethanolic extracts and extract pools showed variations in their compositions, which is related to the extraction method. Sterols, coumarins, and terpenes, including betulinic acid, urechitol A, and urechitol B, have been isolated from *P. andrieuxii* (Yampuc *et al.*, 2009; Domínguez-Carmona *et al.*, 2010; Pan *et al.*, 2012). A more in-depth phytochemical analysis of the phytochemical composition of *Pentalinon* sp. will be carried out in subsequent studies.

Table 4. Yields of the extracts obtained from *Pentalinon* sp.

Extract	Yield (%)
Pentalinon sp. root ethanol	14.45
Pentalinon sp. stem ethanol	12.54
Pentalinon sp. leaf ethanol	18.92
Pentalinon sp. root pool	n/d
Pentalinon sp. stem pool	n/d
Pentalinon sp. leaf pool	5.44

n/a: no data, the final extract was not weighed

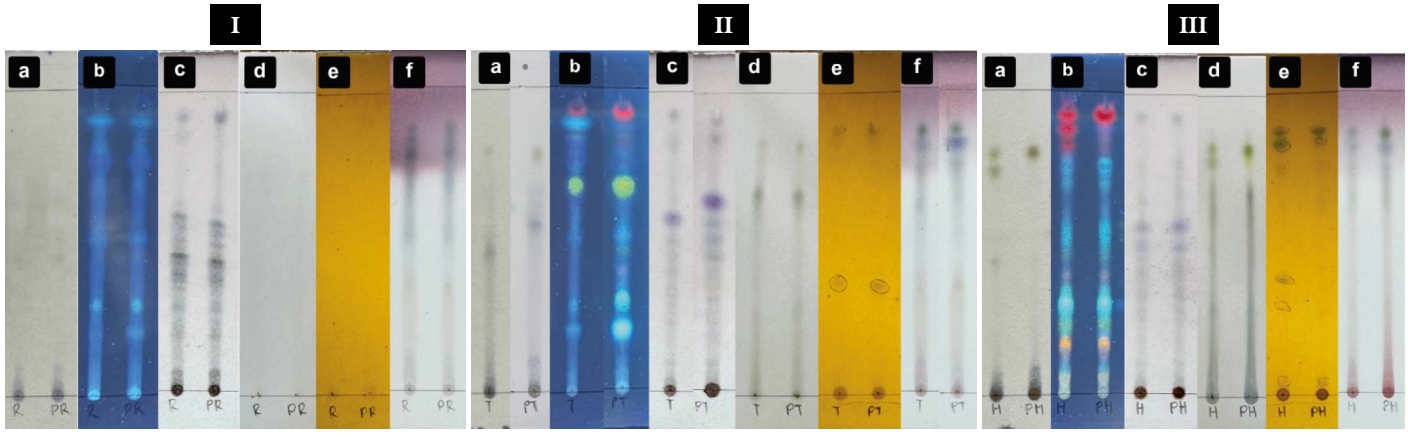

Figure 1. Chromatographic plates for the presence of a: phenols, b: flavonoids, c: terpenoids, d: tannins, e: alkaloids and f: saponins in extracts of *Pentalinon* sp. I: root, II: stems, III: leaves. R: ethanolic extract of root, T: ethanolic extract of stems, H: ethanolic extract of leaves, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts.

Median lethal dose (LD_{50}) of *Bothrops asper* venom and neutralization of lethality with *Pentalinon* sp. extracts

The LD_{50} of the venom pool was 138.9 (131.5-145.9) μg/mouse. None of the evaluated extract doses neutralized the lethality of $3LD_{50}$ of *B. asper* venom (Figure 2). $3LD_{50}$ of venom was recorded as inducing death in mice between 15 and 18 min post-inoculation. The ethanolic leaf extract at a dose of 20 mg/$3LD_{50}$ delayed death for 3 h. The controls of the ethanolic extracts at a dose of 20 mg/mouse induced signs of toxicity (piloerection, loss of hind limb movement, decreased motor activity, and rapid respiration) for a few hours without causing death. The ethanolic root extract at a dose of 5 mg/mouse did not induce signs of toxicity. The root pool (20 mg mouse^{-1}) caused the death of the mice. Of the three doses of the leaf pool evaluated (20, 50, and 80 mg), none showed toxicity like the ethanolic extract. At 20 mg/$3LD_{50}$, the leaf extract pool delayed death by one hour. The 80 mg/$3LD_{50}$ dose did not prevent mortality 18 min post-inoculation.

People who report benefits from consuming this plant may be experiencing multiple factors that are difficult to monitor. These include the amount of venom injected by the snake, the possibility of dry bites, and the presence or concentration of metabolites that may interfere with the lethality of the venom. Furthermore, aspects such as the harvest time and the phenological stage of the plant could also influence the results observed when using *Pentalinon* sp. as an antivenom.

It has been documented that, during reproductive periods, plants tend to synthesize a greater quantity of bioactive compounds (Pires-Moreira *et al.*, 2024). However, none of the plants collected for this study were at that stage. On the other hand, the observed toxicity

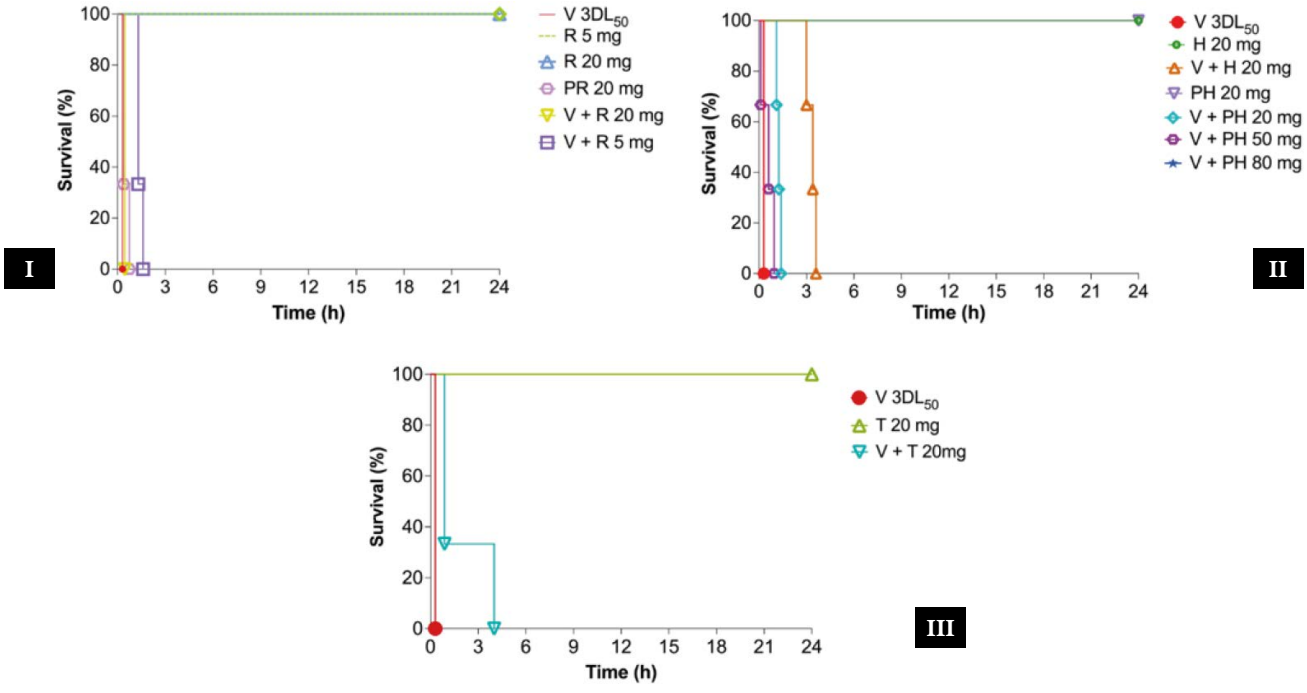

Figure 2. Percentage of survival of mice (ICR) injected with $3LD_{50}$ of *B. asper* venom and *Pentalinon* sp. extracts. I: root, II: leaves, III: stems. V: *B. asper* venom, R: ethanolic root extract, T: ethanolic stem extract, H: ethanolic leaf extract, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts.

could be due to the presence of certain toxic terpenoids. According to Agus (2021), some monoterpenes have cytotoxic effects that vary depending on the dose and exposure time. This coincides with what was observed in the root extracts of *Pentalinon* sp., where 20 mg of the root pool did induce mortality, probably due to a higher concentration of these compounds resulting from the extraction method used. Similarly, it is possible that people who use this plant do not experience adverse effects because the dose used does not contain sufficiently high concentrations of these toxic compounds.

Inhibition of proteolytic activity on azocasein

The proteolytic activity on the venom's azocasein, associated with SVMPs, was significantly affected by the evaluated extracts at different percentages ($P<0.05$). The ethanolic leaf extract inhibited 100% of the proteolytic activity, while extracts PR (43.12±3.46%) and T (45.68±4.71%) had similar inhibition percentages, as did R (14.73±8.54%) and PT (10.16±2.7%). The leaf pool showed practically no inhibition (3.6±2.08%) (Figure 3). SVMPs are enzymes that depend on metal ions such as zinc to perform their catalytic functions (Adrião *et al.*, 2022).

Some flavonoids, such as pinostrobin, have the ability to chelate metal ions (Gómez-Betancur *et al.*, 2014; Kasprzak *et al.*, 2015) and have been shown to partially inhibit the proteolytic and hemorrhagic activity of *B. asper* venom in *in vitro* studies. The phytochemical profile of the roots, stems, and leaves of *Pentalinon* sp. revealed that all organs have a significant flavonoid content, which could be responsible for the inhibition of proteolytic activity. Differences in the phytochemical composition of ethanolic extracts and extract pools were also observed, which may be related to the varying percentages of inhibition among extracts from the same organ.

Inhibition of phospholipase A_2 (PLA2) activity

Of all the extracts evaluated, the ethanolic leaf extract was the only one to partially inhibit phospholipase A_2 activity. Root and stem extracts mixed with the venom generated

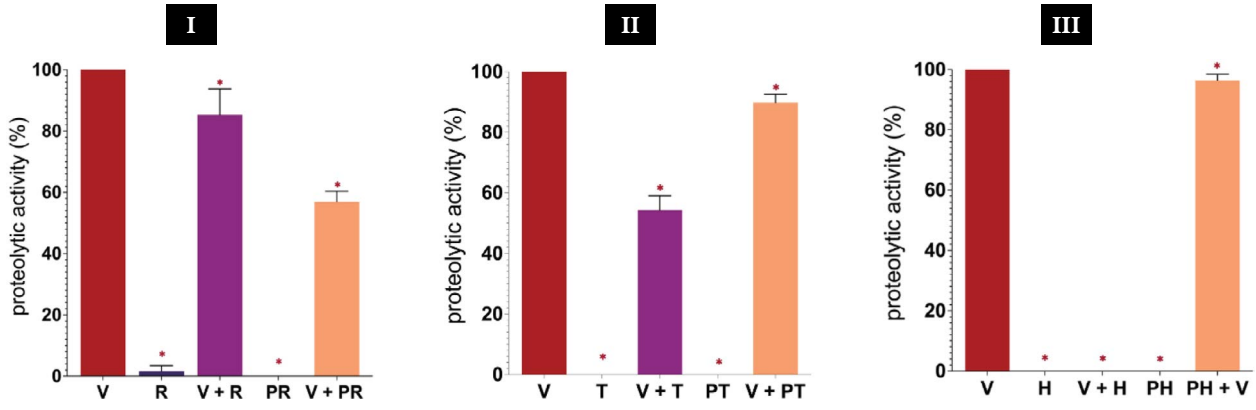

Figure 3. Inhibition of the proteolytic activity of azocasein in *B. asper* venom with extracts of *Pentalinon* sp. I: root, II: stems, and III: leaves. V: *B. asper* venom, R: ethanolic root extract, T: ethanolic stem extract, H: ethanolic leaf extract, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts. Results expressed as a percentage of activity, considering the venom as 100%. Statistical significance ($p<0.05$) compared to the venom: *.

slightly larger halos than those generated by the venom alone (Table 5, Figure 4). The decrease in phospholipase A2 activity can be attributed to the interaction of phospholipase A_2 with components of the extract. Some phenolic compounds, flavonoids, coumestans, alkaloids, steroids, terpenoids, and tannins are among the main plant derived inhibitors of PLA2 (Carvalho *et al.*, 2013).

The phytochemical profile analysis shows that the leaves of *Pentalinon* sp. Some of these groups of compounds are present, which could act by preventing access to the hydrophobic channel for fatty acids, or by binding to functional sites on proteins, or by inducing protein oligomerization (Salvador *et al.*, 2019). In recent years, the search for molecules that inhibit specific venom toxins has gained increasing interest (Salvador *et al.*, 2019), and plants are an important reservoir of such molecules. Future research will need to identify the compounds present in the leaves of *Pentalinon* sp. that interact with phospholipases A_2 and how they interact.

Table 5. Phospholipase A_2 activity of *B. asper* venom and mixtures of venom with *Pentalinon* sp. extracts.

Treatment	Activity phospholipase A_2 (%)
Bothrops asper venom	100
Venom+Ethanolic root extract	108.8±6.505
Venom+Pool of root extracts	101.8±2.512
Venom+Ethanolic stem extract	110.5±8.23
Venom+Pool of stem extracts	104.2±4.149
Venom+Ethanolic leaf extract	60.78±6.618*
Venom+Pool of leaf extracts	95.44±2.702

Values corresponding to the percentage of PLA2 activity (± SD). Results of the average of three repetitions, corresponding to the 1:623 poison:extract (w:w) mixture. *: statistical significance of $p<0.05$ compared with the poison alone (100% activity).

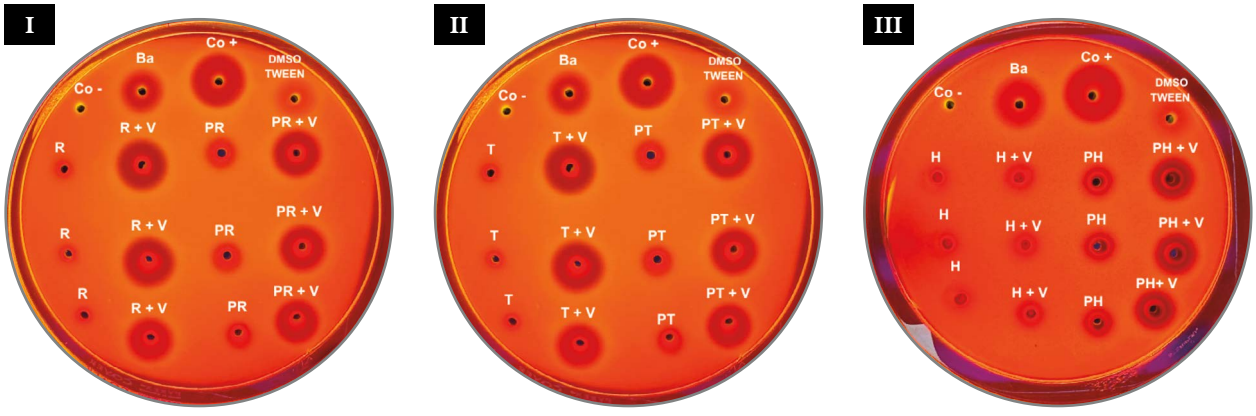

Figure 4. Inhibition of phospholipase A2 activity of *Bothrops asper* venom with extracts of *Pentalinon* sp. I: root, II: stems, III: leaves. Co-: PBS negative control, Co^+: Micrurus tener venom positive control, Ba: *B. asper* venom, V: *B. asper* venom, R: ethanolic root extract, T: ethanolic stem extract, H: ethanolic leaf extract, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts.

Inhibition of fibrinogenolytic activity

The extracts of *Pentalinon* sp. acted in different ways against fibrinogen and venom. Figure 5A, corresponding to ethanolic extracts, shows how the root extract did not interfere with fibrinogen; all three chains remained intact. On the other hand, the stem and leaf extracts induced fibrinogen degradation, as the bands were faded. The ethanolic root extract partially inhibited the degradation of the fibrinogen β band caused by the venom; the ethanolic leaf extract plus venom induced the degradation of all three fibrinogen chains as well as venom components; no bands were observed; and the mixture of ethanolic stem extract with venom induced the same banding pattern as the venom alone (Figure 5A). The pools of stem and leaf extracts did not alter the α, β, and γ chains of fibrinogen. On the other hand, the pool of root extracts maintained all three chains, but they were diffuse (Figure 5B). Regarding the inhibition of fibrinogenolytic activity caused by the venom, although the pool of root extracts interacted with fibrinogen, it only partially inhibited fibrinogenolytic activity; the α and β bands were observed, but not entirely intact.

The stem pool prevented the degradation of the β chain, and the leaf pool showed no inhibition. To determine if the inhibition of chain degradation was better with higher doses, double the dose of root extracts (Figure C) and leaf extracts (Figure D) was evaluated. Root extracts partially inhibited fibrinogenolytic activity, as the α and β bands of the fibrinogen

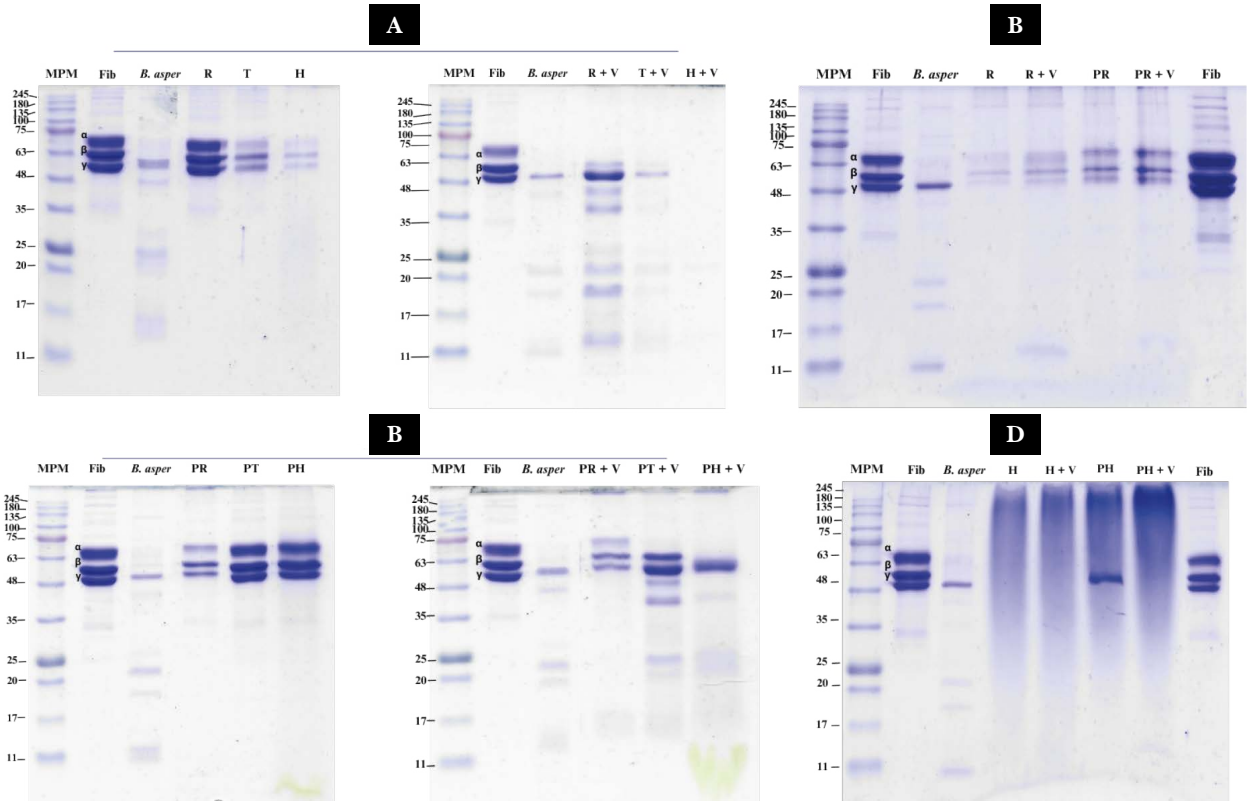

Figure 5. Inhibition of the fibrinogenolytic activity of *B. asper* venom with *Pentalinon* sp. extracts. A) ethanolic extracts, B) pool of extracts, C) root extracts, D) leaf extracts. MPM: molecular weight marker, Fib: fibrinogen as a negative control, *B. asper*: venom as a positive control, R: ethanolic root extract, T: ethanolic stem extract, H: ethanolic leaf extract, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts.

chains, which the venom completely degrades, were faintly visible. With leaf extracts, the fibrinogen and venom bands were lost, showing runs along the gel. Based on these results, intermediate doses between those evaluated may show better inhibition. These results also reflect the different compositions of the extracts, which cause non-homogeneous inhibition. Roots have components that interact with venom enzymes, interfering with fibrinogenolytic activity. The search for inhibitors of venom serine proteinases is limited (Gutiérrez *et al*., 2021). In future research it would be important to evaluate whether inhibition acts in the same way in coagulation assays, since the degradation of fibrinogen chains affects the transformation of fibrinogen to fibrin, which is the protein that forms the clot network (Park and Park, 2024).

This study represents an advance in the exploration of the antivenom properties of *Pentalinon* sp. Although the evaluated extracts did not inhibit the lethality of *Bothrops asper* venom, the presence of bioactive compounds, such as phenols, flavonoids, terpenoids, and saponins, was identified. These compounds interact *in vitro* with venom enzymes, achieving partial inhibition of proteolytic, phospholipase A_2, and fibrinogenolytic activities. Among the plant parts evaluated, the leaves stood out for exhibiting the greatest inhibitory capacity against proteolytic and phospholipase A_2 activities. Significant differences in phytochemical composition were detected between the ethanolic extracts and the extraction pools, which likely explains the variations in their neutralizing capacity. These results underscore the importance of conducting a comprehensive phytochemical analysis to identify the compounds responsible for these interactions and to further their structural and biological characterization.

It is important to emphasize that this study does not propose the use of *Pentalinon* sp. as a substitute for antivenoms, which remain the treatment of choice for snakebites. Instead, this work is positioned as a preliminary analysis aimed at identifying compounds that may complement current treatments, with the goal of addressing the limitations and challenges faced by patients affected by these envenomations. Although the extracts showed partial inhibition of certain enzymatic activities, this should not be interpreted as an indicator of in vivo efficacy. The results have demonstrated that these extracts do not neutralize the lethal activity of *Bothrops asper* venom. Furthermore, toxicity studies revealed that the pool of root extracts, administered at a dose of 20 mg, caused the death of mice, highlighting the need for more detailed toxicological analyses to evaluate their safety.

CONCLUSIONS

This study provides novel information on the potential interactions between extracts of *Pentalinon* sp. and *Bothrops asper* venom. To date, their efficacy and safety for use in treating snakebites have not been demonstrated. These findings open new lines of research, focused both on identifying specific compounds and on their preclinical evaluation, to determine their potential as complementary tools in the management of snake envenomation.

ACKNOWLEDGMENTS

The author, Edgar Enrique Neri Castro, sincerely thanks the SECIHTI's "Researchers for Mexico" program for its support, under which he is conducting research for the 2024 project. The authors express their

gratitude for all the support provided, especially to Felipe Olvera Rodriguez, Alejandro Olvera Rodriguez, Armando Pérez Robles, Audrey Michelle Rodriguez Solis, Emiliano Vazquez Garcia, Arlen Naomi Mompala De León, Fatima Sánchez Ramírez, Luz Adela Guerrero Lagunes, Jonathan Arrollo, Nallely Morales Capellan, Leonardo Fernandez Badillo, and Andrea Jiménez Marín of the Institute of Biology, UNAM.

REFERENCES

Adrião, A. A., Dos Santos, A. O., de Lima, E. J., Maciel, J. B., Paz, W. H., da Silva, F. M., & Koolen, H. H. (2022). Plant-derived toxin inhibitors as potential candidates to complement antivenom treatment in snakebite envenomations. *Frontiers in Immunology*, 13, 842576. https://doi.org/10.3389/fimmu.2022.842576

Agus, H. H. (2021). Terpene toxicity and oxidative stress. In Toxicology (pp. 33-42). Academic press. https://doi.org/10.1016/B978-0-12-819092-0.00004-2

Alape-Girón, A., Sanz, L., Escolano, J., Flores-Díaz, M., Madrigal, M., Sasa, M., & Calvete, J. J. (2008). Snake venomics of the lancehead pitviper *Bothrops asper*: geographic, individual, and ontogenetic variations. *Journal of proteome research, 7*(8), 3556-3571. https://doi.org/10.1021/pr800332p

Bénard-Valle, M., Neri-Castro, E. E., Boyer, L., Jackson, T. N. W., Sunagar, K., Clarkson, M., & Fry, B. G. (2015b). Ineffective traditional and modern techniques for the treatment of snakebite. In B. Fry (Ed.), Venomous Reptiles and Their Toxins: Evolution, Pathophysiology and Biodiscovery (pp. 73-88). Oxford University Press.

Carvalho, B. M. A., Santos, J. D. L., Xavier, B. M., Almeida, J. R., Resende, L. M., Martins, W., & Marchi-Salvador, D. P. (2013). Snake venom PLA2s inhibitors isolated from Brazilian plants: synthetic and natural molecules. *BioMed Research International, 2013*(1), 153045. https://doi.org/10.1155/2013/153045

Domínguez-Carmona, D. B., Escalante-Erosa, F., García-Sosa, K., Ruiz-Pinell, G., Gutierrez-Yapu, D., Chan-Bacab, M. J., & Peña-Rodríguez, L. M. (2010). Antiprotozoal activity of betulinic acid derivatives. *Phytomedicine, 17*(5), 379-382. https://doi.org/10.1016/j.phymed.2009.08.002

Gómez-Betancur, I., Benjumea, D., Patiño, A., Jiménez, N., & Osorio, E. (2014). Inhibition of the toxic effects of *Bothrops asper* venom by pinostrobin, a flavanone isolated from *Renealmia alpinia* (Rottb.) MAAS. *Journal of Ethnopharmacology, 155*(3), 1609-1615. https://doi.org/10.1016/j.jep.2014.08.002

Gutiérrez, J. M., Avila, C., Rojas, E., & Cerdas, L. (1988). An alternative *in vitro* method for testing the potency of the polyvalent antivenom produced in Costa Rica. *Toxicon, 26*(4), 411-413. https://doi.org/10.1016/0041-0101(88)90010-4

Gutiérrez, J. M., & Rucavado, A. (2000). Snake venom metalloproteinases: their role in the pathogenesis of local tissue damage. *Biochimie, 82*(9-10), 841-850. https://doi.org/10.1016/S0300-9084(00)01163-9

Gutiérrez, J. M., Escalante, T., & Rucavado, A. (2009). Experimental pathophysiology of systemic alterations induced by *Bothrops asper* snake venom. *Toxicon, 54*(7), 976-987. https://doi.org/10.1016/j.toxicon.2009.01.039

Gutiérrez, J. M., Albulescu, L. O., Clare, R. H., Casewell, N. R., Abd El-Aziz, T. M., Escalante, T., & Rucavado, A. (2021). The search for natural and synthetic inhibitors that would complement antivenoms as therapeutics for snakebite envenoming. *Toxins, 13*(7), 451. https://doi.org/10.3390/toxins13070451

Kasprzak, M. M., Erxleben, A., & Ochocki, J. (2015). Properties and applications of flavonoid metal complexes. *RSC Adv.* 5, 45853-45877. https://doi.org/10.1039/C5RA05069C

Letsiou, S., Madesis, P., Vasdekis, E., Montemurro, C., Grigoriou, M. E., Skavdis, G., & Tzakos, A. G. (2024). DNA Barcoding as a plant identification method. *Applied Sciences, 14*(4), 1415. https://doi.org/10.3390/app14041415

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of toxicology*, 54, 275-287. https://doi.org/10.1007/BF01234480

Mora-Obando, D., Lomonte, B., Pla, D., Guerrero-Vargas, J. A., Ayerbe-González, S., Gutiérrez, J. M., ... & Calvete, J. J. (2023). Half a century of research on *Bothrops asper* venom variation: biological and biomedical implications. *Toxicon*, 221, 106983. https://doi.org/10.1016/j.toxicon.2022.106983

Neri-Castro, E. E., Bénard-Valle, M., Gil, G., Borja, M, López de León, J., y Alagón, A. (2020). Serpientes venenosas en México: una revisión al estudio de los venenos, los antivenenos y la epidemiología. *Revista Latinoamericana de Herpetología, 3*(2), 5. https://doi.org/10.22201/fc.25942158e.2020.2.205

Ocegueda, S., Moreno, E., & Koleff, P. (2005). Plantas utilizadas en la medicina tradicional y su identificación científica. *Biodiversitas*, 62, 12-15.

Otero, R., Núñez, V., Jiménez, S. L., Fonnegra, R., Osorio, R. G., Garcia, M. E., & Diaz, A. (2000). Snakebites and ethnobotany in the northwest region of Colombia: Part II: neutralization of lethal and enzymatic

effects of *Bothrops atrox* venom. *Journal of Ethnopharmacology, 71*(3), 505-511. https://doi.org/10.1016/S0378-8741(99)00197-X

Pan, L., Lezama-Davila, C. M., Isaac-Marquez, A. P., Calomeni, E. P., Fuchs, J. R., Satoskar, A. R., & Kinghorn, A. D. (2012). Sterols with antileishmanial activity isolated from the roots of *Pentalinon andrieuxii*. *Phytochemistry*, 82, 128-135. https://doi.org/10.1016/j.phytochem.2012.06.012

Park, S., & Park, J. K. (2024). Back to basics: the coagulation pathway. *Blood research, 59*(1), 35. https://doi.org/10.1007/s44313-024-00040-8

Pires-Moreira, M., Chan-Poot, S., Avilés-Berzunza, E., Garcia-Sosa, K., Godoy-Hernández, G., & Peña-Rodríguez, L. M. (2024). Influence of the flowering stage in the production of urechitol A in *Agrobacterium rhizogenes*-transformed plants of *Pentalinon andrieuxii*. *Botanical Sciences, 102*(2), 438-446. https://doi.org/10.17129/botsci.3399

Pérez-Bautista, I., Vilchis-Mercado, I., Galaviz-Mauricio, D. C., Martínez-Betancourt, S. R., Sánchez-Sánchez, Z. S., Sánchez-Cortez, K., & del Mar Ruiz-Posadas, L. (2023). Desarrollos tecnológicos para el aprovechamiento industrial y farmacológico de la contrayerba (*Pentalinon andrieuxii* (Müll. Arg.) Hansen & Wunderlin. *Agro-Divulgación, 3*(3). https://doi.org/10.54767/ad.v3i3.215

Pulido, S. M. T. y Serralta, P. L. (1993). Lista anotada de las plantas medicinales de uso actual en el Estado de Quintana Roo, México. Centro de Investigaciones de Quintana Roo, Chetumal Quintana Roo, México.

Salvador, G. H., Gomes, A. A., Bryan-Quirós, W., Fernández, J., Lewin, M. R., Gutiérrez, J. M., & Fontes, M. R. (2019). Structural basis for phospholipase A2-like toxin inhibition by the synthetic compound Varespladib (LY315920). Scientific Reports, 9(1), 17203. https://doi.org/10.1038/s41598-019-53755-5

Saravia-Otten, P., Hernández, R., Marroquín, N., García, G., Nave, F., Rochac, L., y Gutiérrez, J. M. (2021). Inhibición de las actividades proteolítica y fosfolipasa A_2 del veneno de *Bothrops asper* por el extracto etanólico de *Neurolaena lobata* (L.) Cass. *Ciencia, Tecnología y Salud, 8*(1), 10-23. https://doi.org/10.36829/63CTS.v8i1.865

Sevilla-Sánchez, M. J., Ayerbe-González, S., y Bolaños-Bolaños, E. (2021). Aspectos biomédicos y epidemiológicos del accidente ofídico en el departamento del Cauca, Colombia, 2009-2018. *Biomédica, 41*(2), 314-337. https://doi.org/10.7705/biomedica.5853

Uetz, P., Freed, P, Aguilar, R., Reyes, F., Kudera, J. & Hošek, J. (Eds.). 2024. The Reptile Database. Recuperado el 11 de noviembre de 2024 de http://www.reptile-database.org

Vasquez, C., Neri Castro, E., & Carter, E. D. (2024). Therapeutic itineraries of snakebite victims and antivenom access in southern Mexico. *PLOS Neglected Tropical Diseases, 18*(7), e0012301. https://doi.org/10.1371/journal.pntd.0012301

Wagner, H., & Bladt, S. (2009). Plant drug analysis: a thin layer chromatography atlas. Springer Science. https://doi.org/10.1007/978-3-642-00574-9

Ware, A. G., Guest, M. M., & Seegers, W. H. (1947). Fibrinogen; with special reference to its preparation and certain properties of the product. *Archives of biochemistry, 13*(2), 231-236.

Yam-Puc, A., Escalante-Erosa, F., Pech-Lopez, M., Chan-Bacab, M. J., Arunachalampillai, A., Wendt, O. F., & Peña-Rodriguez, L. M. (2009). Trinorsesquiterpenoids from the root extract of *Pentalinon andrieuxii*. *Journal of natural products, 72*(4), 745-748. https://doi.org/10.1021/np800554n