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Antiedematogenic activity of *Petiveria alliacea* L. in bothropic poisoning

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The biodiversity of the Brazilian biomes inspires interest in research about the biological activities of the molecules present in the flora. In this line, *Petiveria alliacea* a common plant species in the savanna of Tocantins, reported in the treatment for snakebites, was studied to analyze the phytochemical profile and evaluate the activity on the edema caused by *Bothrops moojeni* snake venom. A qualitative phytochemical prospection and a gas chromatography associated with mass spectrometry was performed. Subsequently, it was evaluated whether the extract triggered acute toxicity in Swiss mice (doses of 300 and 2,000 mg/kg), and no toxic effects were observed. Then, the antiedematogenic activity of the extract was performed when administered orally in simulated envenomation with *B. moojeni* snake venom. The presence of bioactive polyphenolics, flavonoids, tannins, coumarins, terpene, alkaloids and saponins compounds was shown. No toxic effects were observed, and the test extract showed evident antiedematogenic activity. It is understood that the inhibition of edema induced by the venom was obtained by reducing the pathways of the inflammatory cascade. Therefore, the traditional effect associated with the plant has scientific basis, in the same way, that its constituents were qualified and showed no toxic effects and has antiedematogenic activity.

Key words: *Petiveria alliacea*, *Bothrops moojeni*, edema, inflammation, snakebite.

INTRODUCTION

Globally, the medicinal use of plants is recognized as an oldest practice, and in many communities, especially when situated in rural areas, and with difficult access, or in underdeveloped countries, it is recognized as a primary source for health care and treatment of diseases. In this context, forest regions are seen with special interest because they represent genuine repositories of biodiversity, ensuring fundamental importance both for survival and for the welfare and health of the communities associated with them (Félix-Silva et al., 2017).

In this sense, the biodiversity contained in the Brazilian biomes and the traditional knowledge about medicinal plants in these communities awaken for the potential for studies involving biomolecules, phytotherapeutics and product development, including medicines (Gutiérrez et

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al., 2021).

Among the natural potential of plants in these biomes, attention is drawn to *Petiveria alliacea*, for being a common plant in several regions of the planet, also present in South America and in the flora of the Savana of Tocantins (Oliveira et al., 2013). This plant belongs to the Phytolaccaceae family, herbaceous plant, rarely arboreal and is preferentially associated with forest environments, easily recognizable by its alliaceous aroma. It is popularly known in Brazil as "*Guinea grass" "Tipi grass", "Amansa-sir"* or *"Garlic Grass"*.

P. alliacea is also used in mystical, esoteric, spiritual, and religious rituals and its popular use also reveals important medicinal potential. Studies highlight chemical substances with important pharmacological properties in the plant, such as alkaloids, sterols, triterpenes, saponins, tannins, lipids and coumarins and cysteine-derived metabolites (Kubec et al., 2002; Duarte and Lopes, 2005).

Other compounds such as the presence of essential oils, especially petiverin, polysulfides, dibenzyltrisulfides, and thiosulfinates are considerably important due to the relevance of these chemical classes in pharmacology in general, and in the production of new drugs (Williams et al., 2007; Gutierrez et al., 2017).

Traditionally, both the aerial parts and the root of this plant are described in several ethnobotanical studies as analgesic, anti-inflammatory, antipyretic, hypoglycemic, antibiotic, antifungal, antitumor, and sedative resources (Lopes-Martins et al., 2002; Santander et al., 2012; Ochoa et al., 2013; García-Peréz et al., 2018). In this line, reaffirming the ethno-pharmaceutical potential of the plant, there are also records on the antiophidic activity and the interesting repellent activity against snakes (Félix-Silva et al., 2017; Trevisan et al., 2021). These data reinforce the importance of continuing investigations into the pharmaceutical properties of this plant.

When it comes to bothropic accidents, they are routine in rural areas and have important clinical repercussions.

Along these lines, bothropic envenomation is usually associated with severe pain at the bite site, inflammation, proteolytic effects, edema formation and hemorrhage. More severe cases involve compartment ischemia, progressing to tissue necrosis, and abscess formation. On the other hand, systemic changes are related to the consumption of factors in the coagulation cascade, production of fibrinolysis, which together reflects in coagulability, and hemorrhage (Fox and Serrano, 2009; Freitas De Sousa et al., 2017; Gutiérrez et al., 2021). These effects trigger important damages, sequels, and losses to the injured, in the same way that they refer to the difficulty and importance of adequate medical care.

It is considered that the inflammatory events triggered and that potentiate the severity in the bothropic accident are dependent on the nature and constitution of the venom, in this line the role of phospholipases (PLA_2) is highlighted, promoting phospholipid hydrolysis of cell membranes, and the mobilization of arachidonic acid, which has repercussions on important myotoxic, inflammatory, and nociceptive activity (Freitas-de-Sousa et al., 2017; Oliveira et al., 2020). These effects are added to the damage produced by serine proteases (SVSP), which trigger a direct conversion of fibrinogen into fibrin, without the need for thrombin, and by metalloproteases (SVMP) that damage endothelial membranes, resulting in coagulopathy and hemorrhage (Gutiérrez et al., 2021).

Classified as a neglected tropical disease, snakebites are an important public health hazard, especially in tropical and subtropical areas. In the Americas, the genus *Bothrops* is responsible for greater part of snakebites, because the wide number of species of this genus. In Brazil the genus *Bothrops* being responsible for majority of the cases reported, and lancehead *Bothrops moojeni*, has great participation because habits various habitats including inhabits the riparian forest and the Brazilian Cerrado biome (Nascimento et al., 2020; dos Santos Cavalcante et al., 2021; Hatakeyama et al., 2021).

Therefore, this study aimed to analyze the phytochemical profile of the plant *P. alliacea* and to evaluate the activity of its extract on the edema caused by the venom of *B. moojeni* snakes.

MATERIALS AND METHODS

Production of crude extract of *P. alliacea*

Samples of *P. alliacea* were collected in the Palmas/TO region, from November 2018 to April 2019, and registered in exsiccate at the Botany Identification Center of the Federal University of in the ratio of 5 g/100 mL of solvent solution, for 72 h. The extract was then filtered and concentrated in a rotary evaporator at reduced pressure, frozen and subjected to complete dehydration in a Terroni® LS 3000 lyophilizer, and then kept frozen at -20 ± 2°C until used in the experiments, based on Simões et al. (2003).

During the experimental protocols, a test solution was made using the freeze-dried crude alcoholic extract and a 20% ethyl alcohol hydroalcoholic water solution as solvent.

Qualitative phytochemical evaluation of *P. alliacea*

Qualitative phytochemical prospection tests were performed to verify the presence of phenols, reducing sugars, alkaloids, flavonoids, tannins, foaming saponins, anthraquinone glycosides, steroids, triterpenoids, and coumarins (Simões et al., 2003; Mouco et al., 2003).

The results obtained were expressed using symbology (+) for minimal traces of evidence of the reaction, (++) for evident traces of observation of the reaction, (+++) for clear evidence of the reaction and enhanced intensity, (++++) for clear evidence of the reaction with strong intensity, and (-) for negative result.

Chromatographic evaluation of the extract of *P. alliacea*

The lyophilized ethanolic extract of *P. alliacea* was analyzed using gas chromatography coupled with mass spectrometry (HS-SPME/GC-MS/MS). The analyses were carried out in a Shimadzu

chromatograph, model QP2020, equipped with an RTX 5 ms column, 0.25 µm thick, 0.25 mm in diameter and 30 m long. The initial programming was at 60°C increasing 10°C per minute to 280°C at a linear velocity flow of 48.9 cm/s and an increase of 3.0 mL/min. Helium was used as carrier gas in the flow in the column of 1.80 mL/min at a pressure of 111.5 kPa.

The identification of compounds was performed by comparison with the mass spectra of the reference library, NIST 107 (National Institute of Standards and Technology Research Library) and Wiley 229 (Panontin et al., 2021). The percentage of components was calculated using the peak area of each substance on the chromatogram.

Toxicity evaluation of the *P. alliacea*

A battery of procedures was carried out respecting ethical principles in animal experimentation, to evaluate lethality and toxicity, with prior approval by the Ethics Committee on the use of Laboratory Animals of the Federal University of Tocantins (nº 23101005186/2018-98) and considering the Guide nº 420 of the Organization for Economic Cooperation and Growth (OECD, 2001), for Chemical Tests - Acute Oral Toxicity, with adaptations and choosing doses of 300 and 2,000 mg/kg for the study, considering the characteristics of this study and of scientific studies like that of García-Pérez et al. (2018).

Swiss mice (*Mus musculus*), male, weighing between 24 and 28 g, were kept in their own boxes and fasted 4 h before the beginning of the experiments, where the water was offered *ad libitum*. The administration done by gavage, the animals from the experimental group received extract at doses of 300 and 2,000 mg/kg, in a 20% hydroalcoholic solution. The control group received a 20% hydroalcoholic solution, without extract.

Toxicity was analyzed based on studies such as Malone (1983), which included observation of animals. In this experiment, the animals were observed during the first 30 min, for 2 min after completing 1, 2, 4, 6, 12, and 24 h (first day), 48 (second day) and 72 h (third day), also on the fifth, eighth, eleventh and fourteenth day after treatment.

At the end of the observation period, the animals were anesthetized with ketamine hydrochloride and xylazine and euthanized by cervical dislocation. For analysis of possible macroscopic changes in the heart, kidneys, lung, liver, spleen, intestine (small and large) and brain were removed and evaluated after the death of the animals according to Vasconcelos et al. (2007) and Santana et al. (2013) with adaptations.

B. moojeni venom

The venom consisted of a pool collected from six snakes. The extracted venom was lyophilized and stored in a freezer at -20°C. The snakes were collected in the state of Tocantins in 2016 and 2017 under the scientific authorization 52416-1, released by the Biodiversity Authorization and Information System (SISBIO), following Normative Instruction 03/2014. The venom proteins were measured according to Smith et al. (1985) by the bicinchoninic acid technique.

The action of the extract on the edematogenic activity

To evaluate the antiedematogenic activity, the pool of bothropic venom (1.8 µg/40 µL sterile saline) was injected into the mice directly into the foot pad, intradermally in one of the hind paws, and the contralateral received the same volume of sterile saline. As a treatment, the hydroalcoholic extract was used at a dose of 1,000 mg/kg, v.o. of *P. alliacea*. The extract was administered by gavage to a group of animals treated 30 min before envenomation, and another group was treated concomitantly with envenomation. The animals in the control group received a 20% hydroalcoholic solution (without the extract). Paw volumes were measured one hour after envenomation with the aid of a caliper, with area calculation performed according to Guzzo et al. (2008) and Santos et al. (2013).

Statistical analysis

The experiments performed minimally in triplicate, had the results expressed as mean ± standard deviation. The data obtained were submitted for statistical analysis using the program SISVAR version 5.6 (Ferreira, 2019). ANOVA analysis of variance ($p < 0.05$) was performed, and the Tukey test or Student T test was applied.

RESULTS AND DISCUSSION

Phytochemical analysis of the ethanolic extract of *P. alliacea* demonstrated the presence of secondary metabolites with various biological activities and considerable pharmacotherapeutic potential. These analyses revealed the presence of reductive sugars, anthraquinones, coumarins, tannins, saponins, terpenes and steroids, in addition to phenolic compounds, flavonoids and alkaloids, shown in Table 1.

The analysis of the chromatographic profile by GC-MS of the alcoholic extract of *P. alliacea* identified 35 main peaks (Figure 1). The identification of these molecular constituents revealed the presence of several phytosterols such as flavonoids (peaks 6, 17, 23, 30), phenolic compounds (peaks 10, 12), fatty acids and esters (peaks 20, 22, 26, 28, 29, 31), benzene compounds (peaks 2, 3, 6, 8, 26), cyclic hexanes (peaks 25), furan (peaks 27), in addition to imidazole compounds (peaks 4, 5), amines (peaks 1, 9, 11, 13, 15, 16, 18), and alkaloid compounds (peaks 14, 15) containing thiols (peaks 19, 23, 26) (Table 2 and Figure 2).

Several studies with *P. alliacea* have already found compounds similar to those detected in this experiment, such as organic acids, phenols, steroids, alkaloids, and saponins. Cruz-Salomón et al. (2022) detected scalene, phytol, and octadecanoic acid, while the studies by Flota-Burgos et al. (2017) and Rosado-Aguiar et al. (2010) detected sulfur-containing organic compounds like those obtained in this study as 9, 12,15-Octadecatrienoic acid, ethyl ester, acetamide, 2-(1,6-dihydro-3-cyano-4-methyl-6-oxo-2-pyridylthio), and thiocyanoic acid, and phenylmethylester.

In this line that, Kubec et al. (2002), also detected similar substances such as N-(4-Tolylsulfonylmethyl) formamide and acetamide, 2-(1,6-dihydro-3-cyano-4-methyl-6-oxo-2-pyridylthio). These substances, important in the survival of the plant in the savanna environment, arouse interest because of their pharmaceutical and medicinal potential.

Different studies have already reported the sedative, anxiolytic and antidepressant activity, of this plant and

Table 1. Qualitative phytochemical screening of hydroalcoholic extract (80%) of *Petiveria alliacea*.

Compound	Test applied	Condition for positivity	Result
Reductive sugars	Benedict's solution	A brick-red precipitate was formed	+
Tannins	Ferric chloride ($FeCl_3$) reaction	Blue precipitate was formed	++
	Acetic-acid and lead acetate reaction	Whitish precipitate formation	++
Saponins	Agitation	Persistent foam	+
Steroids triterpenes	Acetic-acid and ferric chloride ($FeCl_3$) reaction	Ring green or blue was formed	++ (Green)
Phenolic compounds	Folin-Ciocalteau reagent	Precipitate blue color was formed	++++
Flavonoids	Shinoda HCl test and metallic magnesium reaction	Precipitate rose or red was formed	+++ (Red)
Coumarins	Silver Nitrate ($AgNO_3$) reaction	Blackened precipitate was formed	++
	Potassium hydroxide (KOH) reaction	Florescence under ultraviolet lamp	++ (Green)
Anthraquinones	Ammonium hydroxide (NH_4OH) reaction	Precipitate rose or red was formed.	+++ (Rose)
	Sodium hydroxide (NaOH) reaction	Precipitate yellow (reduced) or red (oxidized) was formed	+++ (Red)
Alkaloids	Dragendorff reagent	A brick-red or red orange precipitate was formed	+++ (Red orange)
	Mayer reagent	Precipitate white was formed	+++
	Borchardt/Wagner reagent	Precipitate brown was formed	+++

Source: Trevisan et al. (2022).

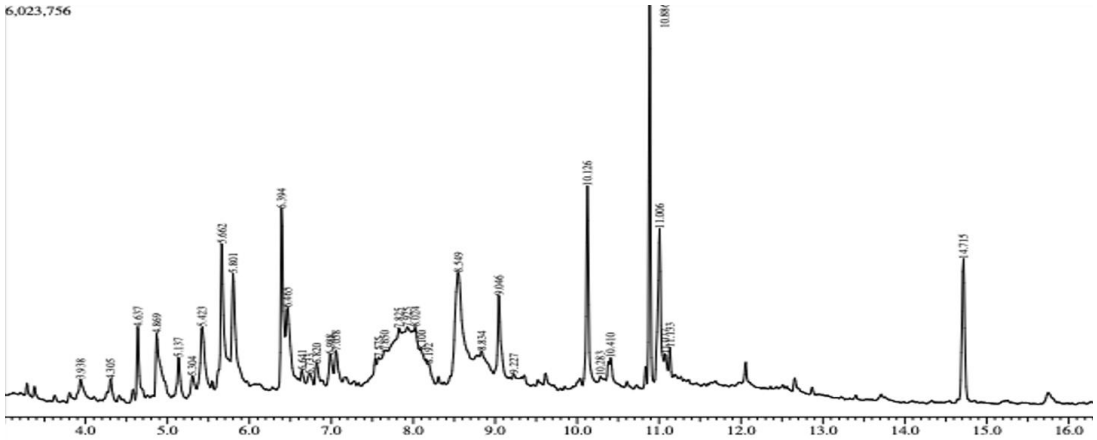

Figure 1. Analysis of hydroalcoholic extract (80%) of *Petiveria alliacea* by Gas Chromatography-Mass Spectrometry (GC-MS).
Source: Trevisan et al. (2022).

Table 2. Retention times and percent yield of compounds identified in hydroalcoholic extract (80%) of *Petiveria alliacea* by gas chromatography-mass spectrometry (GC-MS) analyses.

Peak	Retention Time (RT)	Yield (%)	Identified compounds
1	3.938	*0 .98*	2-Buten-1-amine, N-butyl-, (E)-
2	4.305	*0.58*	Benzeneacetaldehyde
3	4.637	*2.09*	Benzenemethanethiol
4	4.869	*3.91*	4-(4-Methyl-piperazin-1-yl)-1,5, -dihydro-imidazol-2-one
5	5.137	*1.44*	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
6	5.304	*0.96*	Benzoic acid
7	5.423	*3.58*	4-Hydroxy-2-methylpyrrolidine-2-carboxylic acid
8	5.662	*6.61*	Benzofuran, 2,3-dihydro-
9	5.801	*5.30*	4-[2-(Dimethylamino)ethyl] morpholine
10	6.394	*4.90*	2-Methoxy-4-vinylphenol
11	6.465	*3.83*	1-Dimethylaminohexane
12	6.641	*0.96*	Phenol, 2,6-dimethoxy-
13	6.733	*0.62*	1-Aza-9-oxabicyclo [4,4,0] dec-4-en-10-one-2-carboxylic acid
14	6.820	*0.85*	L-Proline, 5-oxo-, methyl ester
15	6.988	*1.17*	Oxazolidine, 2,4,4-trimethyl-
16	7.058	*1.38*	2,3-Dimethylperhydro-1,3-oxazine
17	7.575	*0.90*	5-Benzyloxy-2-nitrotoluene
18	7.650	*1.45*	1-Dimethylaminohexane
19	7.825	*6.43*	N-(4-Tolylsulfonylmethyl) formamide
20	7.925	*3.73*	Hexanedioic acid
21	8.024	*2.12*	3',5'-Dimethoxyacetophenone
22	8.100	*1.39*	Nonanedioic acid, dimethyl ester
23	8.192	*0.62*	Acetamide, 2-(1,6-dihydro-3-cyano-4-methyl-6-oxo-2-pyridylthio)-
24	8.549	*9.94*	Ethyl. alpha. -d-glucopyranoside
25	8.834	*4.77*	6-Ethoxy-6-methyl-2-cyclohexenone
26	9.046	*3.67*	Thiocyanic acid, phenylmethyl ester
27	9.227	*1.10*	2-[5-(2-Oxopropyl) furan-2-yl] propanoic acid, methyl ester
28	10.126	*5.17*	n-Hexadecanoic acid
29	10.283	*0.59*	Pentadecanoic acid, ethyl ester
30	10.410	*1.32*	Benzene propionic acid, 4-benzyloxy-
31	10.886	*7.12*	Phytol
32	11.006	*5.16*	cis, cis, cis-7,10,13 -Hexadecadienal
33	11.077	*0.67*	Octadecanoic acid
34	11.133	*0.54*	9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)-
35	14.715	*4.20*	Scalene

Source: Trevisan et al. (2022).

relate these effects to the presence of compounds such as polyphenols, flavonoids, coumarins, alkaloids and essential oils, highlighting that these compounds were analogous to the compounds that were found in this study (Oliveira et al., 2013; Sathiyabalan et al., 2017). Other studies have also demonstrated important protective effects from oxidation in tissues, and membranes, mainly involving biological capacity of thiols and polyphenol compounds, like the compounds present in this plant (Williams et al., 2007; De Andrade et al., 2012).

The studies also point out that thiol's compounds, flavonoids, tannins, coumarins, terpenic compounds, alkaloids, and saponins classes also detected in the extract of *Petiveria alliacea*, are potent modulators of inflammatory damage, and therefore favor the proposal of this study, where the effects of edema in this type of poisoning are neutralized by the extract. This condition opens the possibility to research the use of coadjutants of this and other plants to the use of the antivenom, collaborating in poison damage protection (Sathiyabalan et al., 2017; Mustika et al., 2021; Chazin et al., 2022).

Accordingly, the analysis of the extract also shows the presence of glycoside compounds, which according to

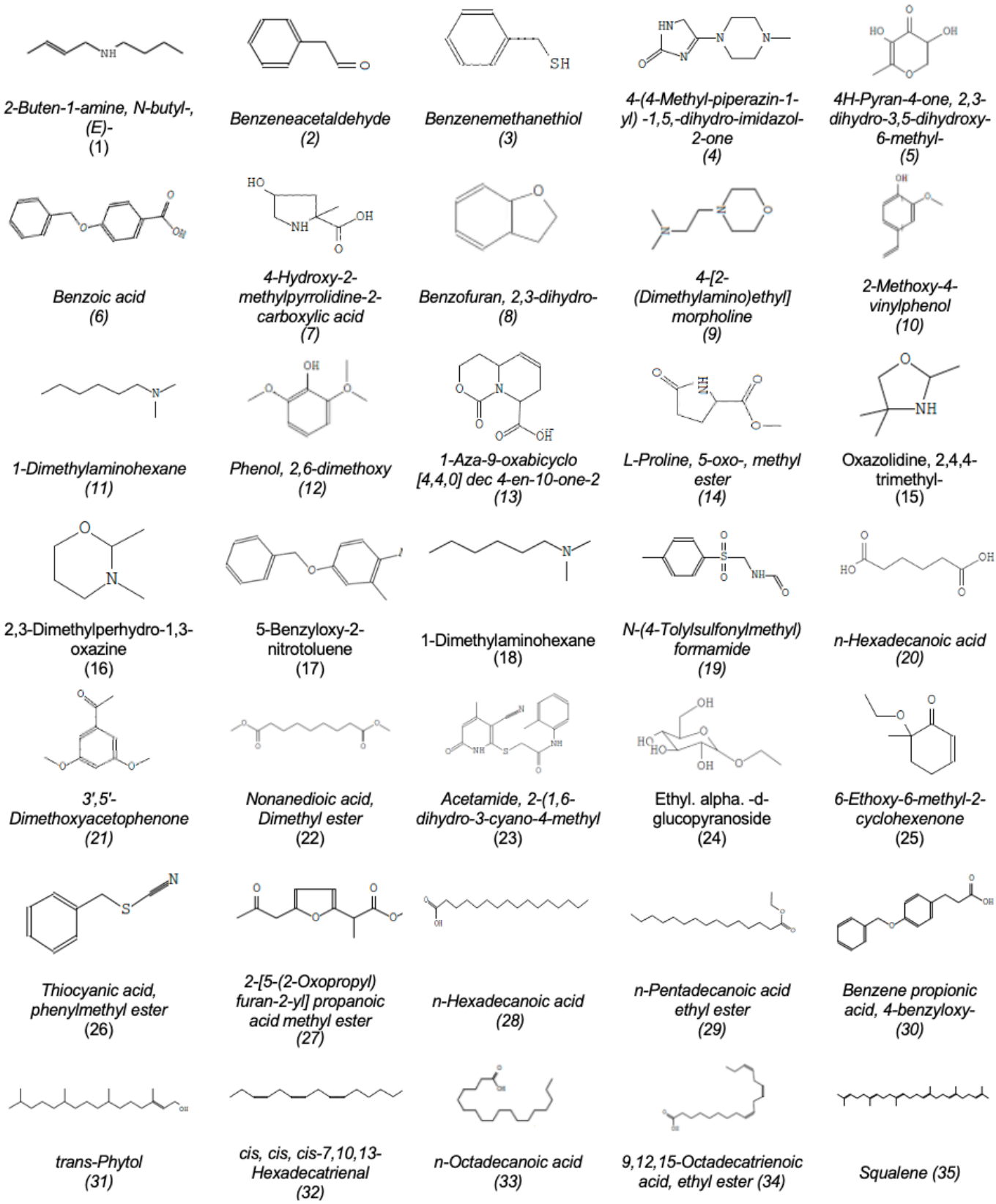

Figure 2. Presentation of the compounds obtained from gas chromatography-mass spectrometry (GC:MS) analysis of the hydroalcoholic extract (80%) of *Petiveria alliacea*.
Source: Trevisan et al. (2022).

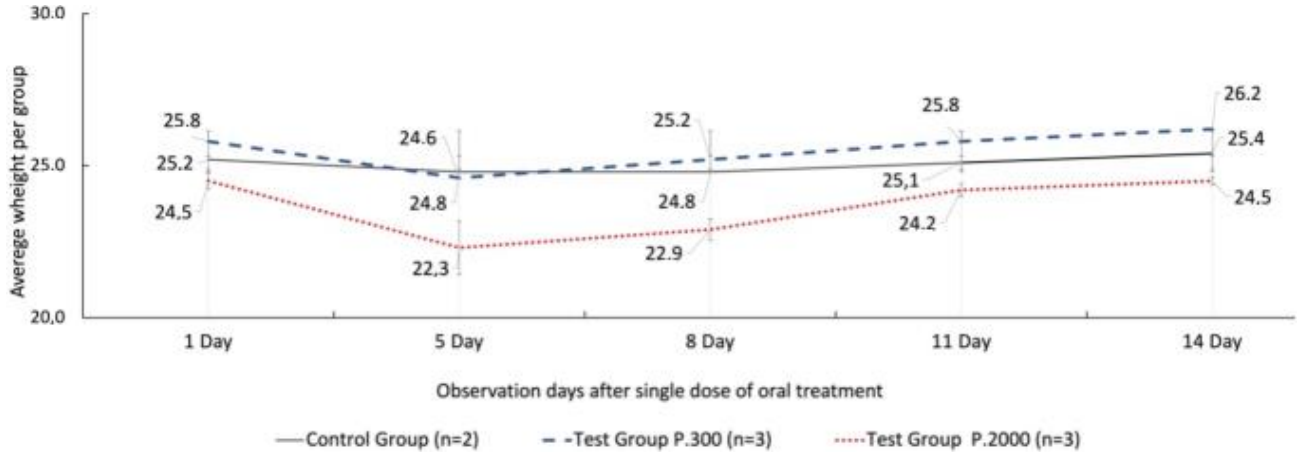

Figure 3. Variation of the average weights of mice observed for 14 days after oral administration in a single dose of the alcoholic extract (80%) of *Petiveria alliacea* Control Group: no treatment, only vehicle. Test Group P.300: treatment with *Petiveria alliacea* extract 300 mg/kg, v.o. Test Group: P.2000, treatment with *Petiveria alliacea* extract 2,000 mg/kg, v.o. *p<0.003; **p<0.002; ***p<0.002, Student t-test.
Source: Trevisan et al. (2022).

Table 3. Coefficient in percentage of organ weight by total body weight, after oral administration in a single dose of the hydroalcoholic extract (80%) of *Petiveria alliacea* and 14 days of observation.

Trail groups	Liver	Heart	Kidney	Lung
Control group	53.48 ± 0.9	6.53 ± 0.2	15.35 ± 1.2	7.34 ± 0.5
Group P. 300	55.34 ± 0.9	6.15 ± 0.2	15.05 ± 1.2	6.44 ± 0.5
Group P. 2000	54.70 ± 0.9	6.08 ± 0.2	17.20 ± 1.2	6.18 ± 0.5
Group test T (Control x group P. 300)	0.759	0.234	0.057	0.071
Group test T (Control group x group P. 2000)	0.134	0.133	0.471	0.053

Control Group: no treatment, only vehicle. Test Group P.300: treatment with *Petiveria alliacea* extracts 300 mg/kg, v.o. Test Group P.2000: treatment with *Petiveria alliacea* extract 2,000 mg/kg, v.o.
Source: Trevisan et al. (2022).

previous studies favor the activity of fibroblasts and collagen produced, as well as hyaluronic acid, that can effectively contribute to reducing tissue damage (Arias et al., 2019). Furthermore, the presence of anthraquinone compounds suggests the possibility of action on macrophages, affecting activation and mediation of acute inflammatory responses, contributing to the anti-inflammatory outcome (Krzak et al., 2021).

However, it is also important to assess the possibility of the plant triggering toxic effects on living organisms (Pedroso et al., 2021), which was also investigated. The description of the acute and lethal toxic effects and macroscopic damage to the organs of the *P. alliacea*, at doses of 300 and 2,000 mg/kg are shown in the Figure 3 and Table 3.

It was also found that the average weight of the mice in the two doses, decreased significantly on the fifth day ($p<0.003$ at the dose of 300 mg/kg, and $p<0.002$ on the fifth and eighth day, at the dose of 2,000 mg/kg), returning to being equal to that on the 8th day for the 300 mg/kg dose and on the 11th day for the 2,000 mg/kgdose. At the end of the test period, on the 14th day, there was no longer any significant difference between the weights of the different groups (Figure 3).

In the evaluation of the toxicity of the extract, there were no deaths at the dose of 300 mg/kg (n=3) nor the 2,000 mg/kg (n=3). The evaluation of the behavioral and physiological parameters (Table 4) did not present any important indication that could show or suggest the toxicity effects of the extract. Thus, it was opportune to continue the study, considering the pharmaceutical potential that bioprospecting revealed, added to the popularly reported antiophidic activity of this plant.

In the behavior analyzed (Table 4), at the dose of 2,000 mg/kg (Test Group P. 2,000), the animals showed a significant change in the parameters of response to touch, tail grip and straightening reflex when compared with the controls. Additionally, during the experiments, a slight decrease in the ambulation of the animals was noticed in the first 3 h of the treatment, and it returned to

Table 4. Behavioral parameters observed for toxicity determination of extracted with hydroalcoholic extract (80%) of *Petiveria alliacea*.

Parameter observed	Group control average	Group P. 300 average	Group P. 2000 average
Response to touch	204	182*	180*
Response to tail squeezing	174	135*	137*
Straightening reflex	253	201*	205*
Body tone	54	55	54
Force to grasp	210	203	201
Auricular reflex	54	55	58
Corneal reflex	156	150	151
Piloerection	3	12	15
Breathing	6	6	7

Criteria such as ataxia, vocal tremor, irritability, contortion, posterior train position, tremors, convulsions, tearing, breathing, and cyanosis were part of the analysis but were not represented in the table because they did not score during the evaluation period. *p<0,005; *ANOVA, Tukey test*.
Source: Trevisan et al. (2022).

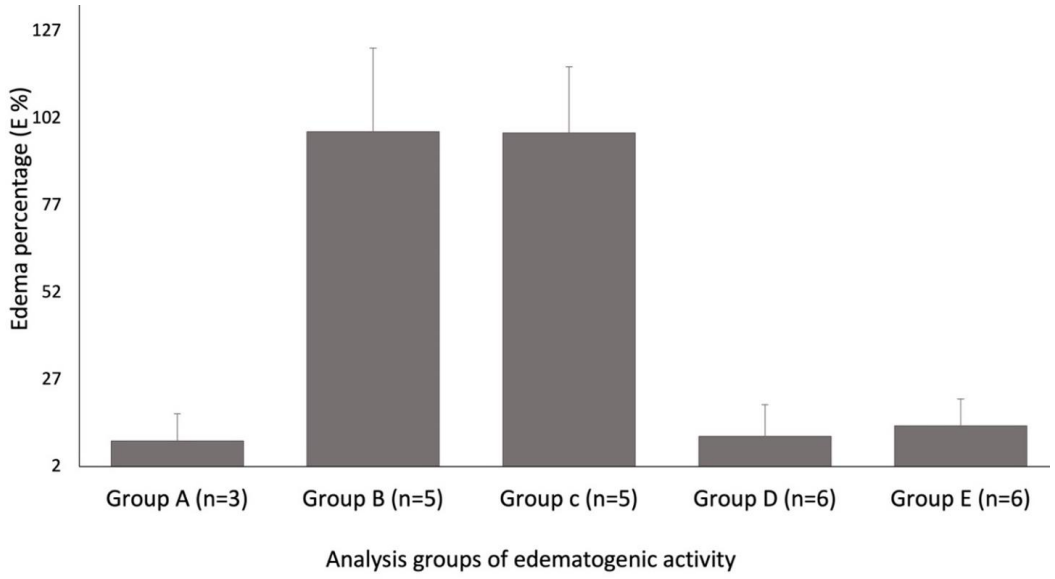

Figure 4. Determination of the paw edema neutralizing activity (E%) of *Petiveria alliacea* (1,000 mg/kg) in bothropic envenomation (1.8 µg of venom). Group A: Normality control (no treatment and no poisoning); Group B: Control with 20% ethanol vehicle, 30 minutes before envenomation; Group C: Control with 20% ethanol vehicle concomitant with poisoning; Group D: Treatment with *Petiveria alliacea* extract 1,000 mg/kg (v.o) 30 minutes before envenomation; Group E: Treatment with *Petiveria alliacea* (v.o) extract (v.o) 1,000 mg/kg concomitantly with envenomation (1.8 µg diluted in 40 µL of sterile saline solution). *p<0.05, Student t-test.
Source: Trevisan et al. (2022).

the normal behavior after 6 h. As this finding did not influence the outcome of the acute toxicological analysis of the extract, there were no measures to expand the investigation.The evaluation of the antiophidic potential of *P. alliacea* in bothropic envenomation highlighted the important antiedematogenic activity. The edema induced by the mixture of *B. moojeni* venoms was inhibited by the extract of *P. alliacea* (1.000 mg/kg), when administered 30 min before envenomation, and when administered simultaneously with envenomation (p<0.01; Figure 4), showing protection and immediate inhibition of edematogenic activity. Furthermore, were observed no difference between these two treatments (p=0.266). These results express the ability of the extract of *P. alliacea* to reduce or even neutralize the edematogenic responses, characteristic of this category of

envenomation.

The results are associated with the existence of the biological activity of the various chemical compounds observed in the chromatographic analysis of the extract and generate expectations about how the antiedematogenic effect occurs. Thus, we work with two hypotheses: the first, this mixture of biomolecules revealed in the present extract acts on the inflammatory cascade, impacting the neutralization of the edematogenic effect of the poisoning, or the second, only a functional group or specific molecule.

Bothropic venom has metalloproteases (SVMPs), serine proteases (SVSPs) and phospholipases (PLA2) which are widely described components in the poisoning process and probably, somehow are affected by the extract, leading to decrease efficacy of their effects. As these compounds are involved in the recruitment of phagocytic cells, in the release of eicosanoids and cytokines, factors that affect the propagation and amplification of inflammatory responses, or the other myotoxic damage and changes in the body's hemostatic system, have conditions to help and promote the reduction of this damage when triggered by poisoning (Mamede et al., 2020).

Therefore, the mechanism of evolution of the edema produced by bothropic envenomation is being neutralized, in some way, in the presence of the extract under test. These effects are due to the set of biochemical actions of inflammatory mediators, tissue damage, and damage to the microvasculature, as well as the result of the release of inflammatory mediators such as histamine, bradykinin, cytosines, eicosanoids, nitric oxide, among others, that can be neutralized by the natural components of the extract. The joint and expanded action of these components lead to the loss of tissue stability, the increase in permeability and, consequently, the activation of inflammatory pathways and edema formation (Mamede et al., 2020).

Arachidonic acid present in the extracellular environment, as a product of the hydrolysis of membrane phospholipids that were metabolized by the venom's PLA_2, is another potential target of the components of the extract under test, as it becomes a substrate in the lipoxygenase and cyclooxygenase pathways. These biochemical pathways catalyze the formation of prostaglandins and thromboxane’s, substances directly involved in the propagation and local and systemic inflammatory responses (Teixeira et al., 2009; Freitas-de-Sousa et al., 2017).

Studies report the important role played by SVMPs, especially those of class PI and PIII present in the venoms of *Bothrops jararaca* and *B. moojeni*, proteolytic classes that also act in addition to the proteolytic effects and act by catalyzing pro-inflammatory agents that amplify the symptoms and aggravate the injury of poisoning (Resiere et al., 2020).

In this sense, the experimental treatment with the extract of *P. alliacea*, by acting to reduce the intensity or impediment of the inflammatory pathways from the snakebite, may even collaborate in the elucidation of the antiophidic activity associated with this plant. Note that the phytochemical and chromatographic profile (Table 1 and Figure 1) revealed the presence of terpenoids, flavonoids, anthraquinones, phenolic compounds, alkaloids and coumarins. The components observed in the extract of this study are related to the already reported anti-inflammatory activity of the plant (Duarte and Lopes, 2005).

Other studies also report biological activities for compounds obtained from chemical extraction in medicinal plants, which have anti-inflammatory, anticoagulant and antioxidant activities associated with the presence of phenolic compounds, flavonoids, alkaloids, coumarins and saponins (Dailey and Vuong, 2015; Guillamón Enrique, 2018). These compounds, obtained from medicinal plants, can interact in metabolism and biochemical pathways involved in inflammatory processes, highlighting the possibility of being inhibitors of phospholipase, cyclooxygenases, lipoxygenases, and of prostaglandins and leukotrienes and cytosines, interleukins (Adrião et al., 2022).

The presence of compounds based on terpenoids and polyphenols collaborate with the expectation of inhibitory effects on oxidative stress in tissues, involving the reduction of the synthesis of interleukins and pro-inflammatory cytokines, including the action of phospholipase A_2 which affects the lower generation of free radicals and consequently less damage to the phospholipid membrane, as suggested by Działo et al. (2016) and Muthusamy et al. (2017).

Furthermore, the tannins, saponins, alkaloids, and flavonoids that the *P. alliacea* extract presented in its constitution are biomolecular classes that have been shown to have precipitating activity on proteins and that contribute to promoting tissue-protective activities (Samy et al., 2012).

This information raises the possibility that the antiedematogenic activity observed in this study is associated with the precipitating capacity of these compounds on venom proteins, reducing their effectiveness and impacting the effectuation of the neutralizing effect of edema experimentally observed.

The extract of *P. alliacea* against the fibrinogen activity, hemorrhagic, and myotoxic activities induced by the venom of *B. moojeni* was also studied, but it was not effective in neutralizing them. What was noticed is that bothropic envenomation occurred in an expected manner and in agreement with other studies that dealt with the effects of this envenomation (Oliveira et al., 2020).

Conclusion

Based on the results of this study, it is possible to

conclude that the extract of *P. alliacea* did not present deleterious effects at the doses used, justifying the continuity of investigations to understand the popular use in the treatment of bothropic snakebites. The results were satisfactory to control the edema induced by bothropic venom, in paw of mice. Therefore, it is possible to infer that the antiedematogenic results observed in the extract occurs through the physiological mediation of the various compounds present in the extract on the inflammation pathways triggered by the protein material of the venom. Finally, once the modulation of the inflammatory response was successfully achieved, further studies should be implemented to understand the mechanisms and determine which molecules or groups of compounds are responsible for this activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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