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Inhibition of the principal enzymatic and biological effects of the crude venom of *Bothrops atrox* by plant extracts

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This study analyzes the effectiveness of extracts from five plant species used for treating venomous snakebites in the region of Santarém, in central Brazilian Amazonia. The aqueous extracts of *Bellucia dichotoma* Cogn., *Connarus favosus* Planch., *Aniba fragrans* Ducke, *Plathymenia reticulata* Benth., and *Philodendron megalophyllum* Schott were prepared by decoction at 70°C and lyophilized. Inhibition of the enzymatic activity and biological effects of the crude venom of *Bothrops atrox* was tested by pre-incubating different concentrations (1:1 to 1:30 venom:extract, w/w) for 30 min at 37°C. These results confirm traditional local knowledge on the anti-toxin powers of the native plants of the Amazon basin and the potential of these extracts as toxin inhibitors and as an alternative or complement for the standard treatment of snakebite based on the application of antisera.

Key words: Natural inhibitors, *Bothrops atrox*, *Bellucia dichotoma, Connarus favosus, Plathymenia reticulata, Philodendron megalophyllum, Aniba fragrans.*

INTRODUCTION

Poisoning by the venom of *Bothrops* vipers is characterized by both local and systemic effects. The local effects normally include pain, hemorrhage, edemas, myonecrosis and inflammation on the site of bite, while systemic problems include alterations in blood clotting (consumptive coagulopathy), cardiovascular and renal alterations, hypovolemic shock, and hemorrhaging in other parts of the body (Warrell, 2004; Gutiérrez and Rucavado, 2000; Gutiérrez et al., 2005). These consequences arise soon after the bite and result from

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the combined effects of the different toxins contained in the venom.

Snake venoms are complex combinations of proteins, in which hundreds of different molecules can be detected using bidimensional electrophoresis (Valente et al., 2009). The principal toxins are either enzymatic, such as serinoproteinases, metalloproteinases, L-aminoacid oxidases and A_2 phospholipases, or non-enzymatic substances, which include disintegrins, type-C lectins, myotoxic peptides, neurotoxins, cytotoxins, and bradykinin potentializers (Calvete et al., 2009). As mentioned above, the principal physiological targets of these toxins are components of the hemostatic system or the tissue adjacent to the site of the snakebite.

Bothrops atrox, which is known as the "northern viper" in the Brazilian Amazon region, is involved in most cases of venomous snake bite and responsible for approximately 90% of all reported incidents in the region (Cardoso et al., 2009). These cases are normally treated through the parenteral administration of antiserum. While this does neutralize the systemic effects of the venom, local tissue damage is unaffected, so it is important to develop a natural or synthetic treatment that complements the administration of antiserum (Cardoso et al., 2003). Plants with potential anti-toxin effects have stimulated growing interest in the field of bio-active molecules due to their potential use as a complement to traditional antiserum treatment. The potential medical and economic value of many plants has stimulated a great deal of scientific research, including numerous ethnopharmacological studies, which have identified a number of plants with useful pharmacological properties (Da Silva et al., 2007).

Traditional Brazilian medicine is rich in plant-based remedies for the treatment of a wide range of ailments, including snakebite. In the Amazon region, for example, indigenous peoples use macerated leaves of *Pentaclethra macroloba* as an ointment for topical application on the site of bite (Da Silva et al., 2005). Concerning *B. atrox*, it has been reported that extracts from *Marsypianthes chamaedrys* reduced venom induced leukocyte migration (Magalhães et al., 2011), flowers extracts of *Peltodon radicans* inhibited the venom induced edema (Da Costa et al., 2008) and *Humirianthera ampla* extracts reduced 75% of the myotoxic effect of the venom (Strauch et al., 2013). However, the majority of plant species used in Amazonian folk medicine remains without scientific validation.

In the vicinity of the city of Santarém, in the central Brazilian Amazonian basin, five plant species: *Bellucia dichotoma* Cogn., *Connarus favosus* Planch., *Plathymenia reticulata* Benth., *Aniba fragrans* Ducke, and *Philodendron megalophyllum* Schott are used in local communities for the treatment of snakebite and other hemorrhagic problems. The extracts are normally used in the form of ointments or teas, although the effectiveness of these plants as a remedy for the symptoms of snakebite has yet to be evaluated scientifically. The present study is part of the project denominated - "Anti-toxic plants from the Amazon basin - raw material in the quest for new metalloproteinase inhibitors", which investigates traditional knowledge on the use of plant remedies for snakebite in the central Amazon basin.

In this context, the present study evaluated the potential of the aqueous extracts of the five above mentioned species for the inhibition of the principal enzymatic and biological effects induced by the crude venom of *B. atrox.*

MATERIALS AND METHODS

Plant samples and aqueous extracts

The samples of *P. megalophyllum* (liana) and *C. favosus* (bark) were collected in an area of savanna near the village of São Pedro (02° 32' 08.9" S, 54° 54' 23.9" W). *B. dichotoma* (bark) and *P. reticulata* (bark) were collected in the community of Cucuruna (02° 27' 21.0" S, 54° 47' 45.7" W) and *A. fragrans* (leaves) at the Curaua Experimental Farm (02° 33' 99.3" S, 54° 36' 61.2" W). All sites are located in the municipality of Santarem in the Brazilian state of Para, and their coordinates were recorded on a 60 csx Garmin - GPS, Kansas, EUA. Voucher specimens of all the species were deposited in the herbarium of EMBRAPA/eastern Amazon in Belem, Para (Brazil) under catalog numbers (IAN) 185215 for *P. reticulata*, 185216 (*C. favosus*), 184899 (*P. megalophyllum*), 184897 (*A. fragrans*), and 1852213 (*B. dichotoma*).

The samples were sorted, cleaned, and desiccated at 40°C in a Licit LC-E80, forced ventilation oven and macerated in a mortar to obtain grains of approximately 6 mm in diameter. A 100 g sample of the powder of each plant was used for extraction with distilled water at a ratio of 1:5 (w/v) under constant agitation for 2:30 h at 1,250 rpm and a temperature of 70 ± 5°C. Once cooled, the solution was filtered, resulting in approximately 480 ml of aqueous solution, which was then lyophilized (Liotop – L10, Sao Paulo, Brasil). The productivity of this process was estimated based on the dry biomass, and was 10.5% for *P. reticulata*, 6.7% for *C. favosus*, 1.2% for *P. megalophyllum*, 15.4% for *A. fragrans*, and 11.6% for *B. dichotoma*. The lyophilized extracts were stored at 4°C and then resuspended at 2 mg/ml to produce the aqueous extracts (AEs) used in the analyses.

Phytochemical profiles of the aqueous extracts

The phytochemical profiles of the aqueous extracts (AE) were obtained by thin layer chromatography (TLC), using a M & N aluminum support, 5 × 10 TLC F_{254} silica gel plates, ATS4 automatic applicator, a TLC Visualizer, and a 20 × 10 cm bipartite chamber, with data being collected on WinCats 1.4.4 software, all developed by Camag, Muttenz, Switzerland. Four elution systems of different polarities were used according to the metabolite analyzed. The apolar system was composed of hexane:ethyl acetate:formic acid (42.5:7.5:2.5, v/v), the acidic polar system of ethyl acetate:acetic acid:formic acid:water (25:2.75:2.75:6.75, v/v), the alkaline polar system of ethyl acetate:isopropanol:dietylamine

(9:7:4, v/v), and the medium polarity system of hexane:ethyl acetate:formic acid (5:5:1, v/v).

A sample of 10 mg of each lyophilized product was dissolved in 5 ml of methanol and given a 1 min ultrasound bath (Branson® 251), resulting in a new suspension of 2 mg/ml and 10 µl of each AE solution was applied to the chromatoplates. Following elution, the plates were photodocumented in the 366 nm wavelength and then derivatized with specific reagents for the identification of the principal classes of chemical substances present in the extracts – vanillin sulfuric acid (VSA) for the visualization of terpenoids (yellow-brown coloration) and fatty acids (blue), NP-PEG (diphenylboryloxyethylamine/polyethylene glycol) for the visualization of cumarins (blue-green) and flavonoids (yellow-orange), and potassium hydroxide (KOH) for anthrachinones (red-yellow) and cumarins (blue-green). Aesculin, rutin, and thymol (all from Sigma-Aldrich) were also used for the determination of cumarins, flavonoids, and terpenoids, respectively, all at 98% purity and a concentration of 1000 µg/ml.

Animals and venom

Venom was collected from specimens of *B. atrox* obtained at kilometer 83 in the Tapajós National Forest in the municipality of Santarém, Brazil. Specimen collection was authorized by SISBIO license number 14018. The venom was collected *in natura* and lyophilized. Groups of five Swiss mice of both sexes (34 to 41 g) and male Wistar rats (120 to 190 g) were obtained from the animal house of the Federal University of Western Pará in Santarem, Para (Brazil). The animals were kept in standard housing conditions (temperature 22 ± 1°C, 12 h light/dark cycle) with food and water freely available. All the experiments were conducted in accordance with the legal requirements of Brazilian federal law 11.794 of October 8th, 2008 and were approved by the Ethics Committee for the Experimental Use of Animals at Pará State University (UEPA) under protocol 43/11. The crude venom of *B. atrox* was dissolved in saline solution (0.9% NaCl) to 1 mg/ml and the concentration of proteins was determined by Bradford's (1976) method. For the inhibitory experiments, solutions containing a fixed quantity of the venom protein were mixed with different quantities of the AEs, in proportions (venom:inhibitor, w/w) varying from 1:1 to 1:30, depending on whether the test was conducted *in vitro* or *in vivo.* All the mixtures were incubated for 30 min at 37°C and aliquots were tested in four types of assays. The following controls were also done: crude venom plus saline solution (positive control), and AE + saline solution (negative control).

***In vivo* experiments**

Hemorrhagic activity

The hemorrhaging caused by the *B. atrox* venom was assessed using the method described by Gutierrez et al. (1985). The minimum hemorrhagic dose (MHD) was defined as the smallest quantity of venom in micrograms that caused a hemorrhagic lesion of at least 10 mm in diameter after one hour when injected into the shaved dorsal skin of the Swiss mice (n = 5). After one hour, the animals were euthanized and the dorsal tissue was removed and photographed, and the images were digitalized using a HP Deskjey F4280 scanner and saved in RGB format files for processing in a Matlab script (Gonzalez et al., 2009) using Dougherty's (2002) procedure. The different components of the image were selected using a threshold device (Guerra et al., 2011) and a morphological gradient processor to obtain the hemorrhagic halos. The area (mm^2) and greatest diameter of the hemorrhage were measured using the approach of Dougherty and Lotufo (2003). The AEs were pre-incubated for 30 min at 37°C with 2MHD of the venom, then injected as already described and their inhibitory effect on the halo measured.

Edematogenic activity

The edematogenic activity of the crude venom was evaluated using Yamakawa et al. (1976) procedure. For this, the kinetics of the edematogenic activity was initially established. The venom was dissolved in 0.9% NaCl solution and 100 µl was injected into the plantar pad of the right posterior paw (test) of rats. The left posterior paw was injected with an equal quantity of sterile saline solution (control). Both paws were marked with a projector pen in the tibial-tarsal region. Paw volume was measured using a digital EFF 304 plethysmometer immediately after the injections (time zero) and subsequently at intervals of 30 min, and 1, 2, 4, 6, and 24 h. The edema produced by the venom was expressed as the percentage of the increase as related to the control paw volume. The minimum edematogenic dose (MED) of the venom was defined as the smallest quantity necessary to induce a 30% increase in the thickness of the test paw after 1 h. The neutralizing effect of the AEs on the edematogenic activity of the venom was evaluated by incubating 2MED of the crude venom with the AEs (1:5, w:w) for 30 min at 37°C before injections and measuring the thickness of the plantar pads after 1 h.

***In vitro* experiments**

Phospholipase A_2 activity

Inhibition of phospholipase activity was measured indirectly through the hemolytic activity on agarose gel, using egg yolk as phospholipid source and human erythrocytes as the substrate. The minimum indirect hemolytic dose (MIHD) was calculated according to Gutierrez et al. (1988) and considered to be a measure of phospholipase A_2 activity, defined as the quantity of venom that produces an hemorrhagic halo of 10 mm after 24 h. The neutralizing effects of the AEs was evaluated by pre-incubating the extracts with 2HIMD of the crude venom for 30 min at 37°C at ratios of 1:1, 1:2, 1:5, 1:10, 1:20, and 1:30 (w/w) before the assays. Enzymatic activity is expressed as the percentage of inhibition, with a value of 100% corresponding to the complete absence of the hemolytic halo. Each assay was run in triplicate and the results were presented as the mean ± SD.

Coagulatory activity

Coagulatory activity was calculated based on the approach of Assakura et al. (1992), and was expressed as the mean time of coagulation in seconds induced by the crude venom of *B. atrox* in 100 µl of human plasma pre-incubated at 37°C. Coagulatory activity was determined by measuring the time of coagulation to the first sign of the formation of a fibrin network. The minimum coagulatory dose (MCD) was defined as the quantity of venom necessary to coagulate 100 µl of plasma in 60 s.

The neutralizing effect of the AEs on the coagulatory activity of the venom was evaluated by pre-incubating the extracts with the MCD of the crude venom for 30 min at 37°C at the ratios of 1:5,

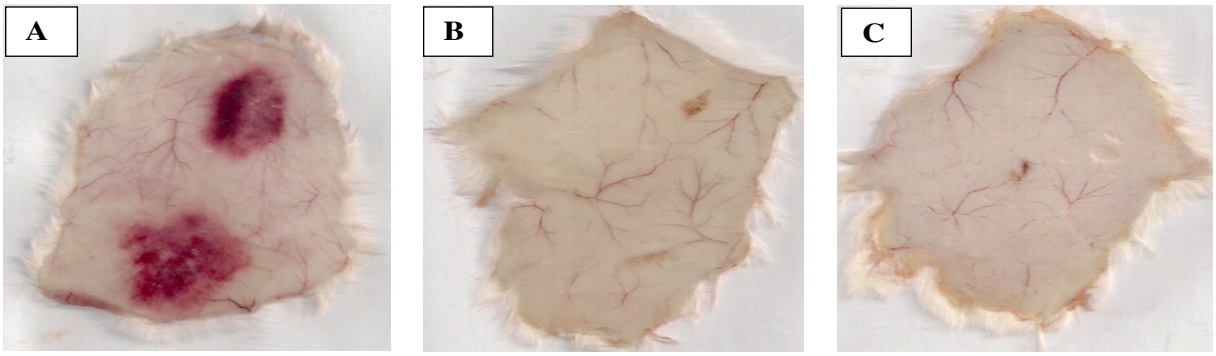

Figure 1: inhibition of the hemorrhage by the AE of *B. dichotoma*. (A) 10 µg of venom; B) 10 µg of venom + 50 µg of the AE of *B. dichotoma* (1:5); C) 10 µg of venom + 100 µg of the AE of *B. dichotoma* (1:10).

1:10, and 1:20 of the MCD to the AE. Each mixture was then added to the plasma (100 µl) and the coagulation was monitored as described above. The total absence of a fibrin network after the maximum interval of 10 min was considered to represent 100% inhibition.

Statistical analysis

The results of the experiments were presented graphically using the Microcal Origin 8.5 program, with all values representing the respective mean ± standard deviation (SD). When appropriate, Student's *t* was applied for the comparison of means, considering $p < 0.05$ as significant.

RESULTS AND DISCUSSION

Phytochemical profile of the aqueous extracts

The results of the phytochemical prospection of the extracts by TLC are summarized in Table 1. Hydrolizable tannins were detected in all the extracts. These tannins appear to be able to ligate to the proteins found in venom during the incubation period in an unspecified fashion (Ambikabothy et al., 2011; Sia et al., 2011), which may account for the high efficiency of inhibition recorded in both the *in vivo* and *in vitro* assays run in the present study. Farrapo et al. (2011) conducted a similar study with *P. reticulata*, one of the target species of this study, showing that the content of polyphenols tannin was 20 times higher than the content of flavonoids (0.16%), concluding that the presence of tannins in *P. reticulata* was able to inhibit the toxic effects of the venom of *B. jararacussu*.

Inhibition of hemorrhagic activity

The hemorrhaging caused by the intradermic injection of *B. atrox* venom in mice was dose-dependent, MHD being defined as 5 µg of the protein present in the venom. The hemorrhagic activity caused by the intradermic injection of 2MHD of the crude venom of *B. atrox* was inhibited completely by the *B. dichotoma* extract when the sample was mixed with the venom prior to the injection at ratios of 1:5 and 1:10 (w/w) (Figure 1). All other AEs inhibited hemorrhagic activity significantly: *C. favosus* by 73.32 ± 7.1 and 94.45 ± 1.6% (1:5 and 1:10, respectively), *A. fragrans* by 81.89 ± 5.4 and 92.24 ± 6.9%, *P. reticulata* by 70.06 ± 6.5 and 85.26 ± 5.5%, and *P. megalophyllum* by 71.77 ± 1.8 and 96.58 ± 5.9% (Figure 2).

The metalloproteases are important enzymes in cases of poisoning and require divalent ions, such as zinc, in order to function (Fox and Serrano, 2008). Given this, inhibition of the metalloproteases may be related to the ligation of the chemical compounds in the AEs with this ion. Tannins and flavonoids appear to be capable of chelating metallic ions such as zinc (Castro et al., 1999). Hydrolizable tannins are present in all the AEs analyzed in this study, although it is not possible to infer which individual metabolite is responsible for the inhibition of hemorrhaging, given the possibility of synergism amongst the different classes of compounds in the extracts.

Inhibition of edematogenic activity

The initial evaluation of the same rats (intra-group) at different times was conducted in order to induce an acute inflammatory reaction and determine the MED. In the case of the positive and negative controls, there was no significant ($p > 0.05$, Student´s *t* test) increase in the thickness of the paws. The MED for the venom of *B. atrox* was 5.0 ± 0.07 µg, which provoked an intense reaction one hour after the injection, although the values had returned to normal after 6 h (Figure 3). Local inflammation is a characteristic of viperid bites, although

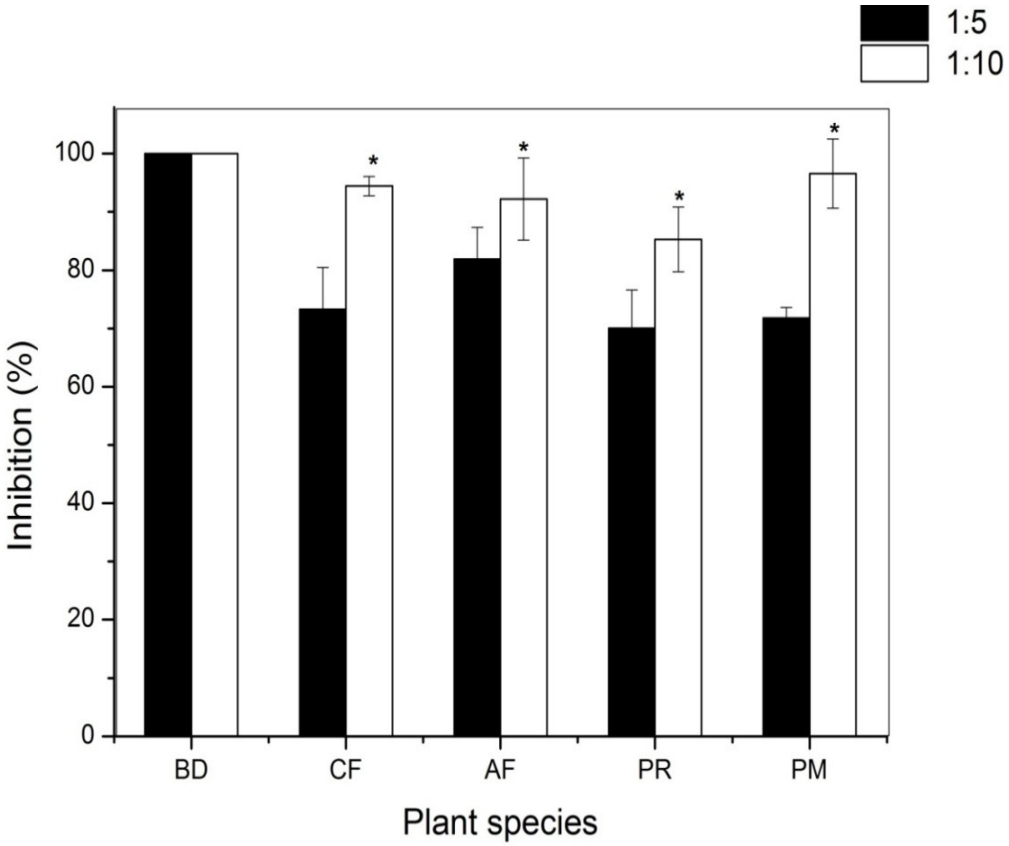

Figure 2: Inhibition of the hemorrhagic activity of the venom of *B. atrox* by the AEs of BD = *B. dichotoma,* CF = *C. favosus,* AF = *A. fragrans*, PR = *P. reticulata*, PM = *P. megalophyllum.* 2 MHD of the venom was pre-incubated at different venom:extract ratios (1:5, black and 1:10, white, w/w). All AEs inhibited significantly (* $p < 0.05$) as compared with the controls. Values are means±SD, n = 5.

it may be possible to treat the local edema caused by the venom of *Bothrops* vipers by vasoactive substances, such as histamines and serotonins, as well as prostaglandins and kinins (Dennis, 1994).

As shown in Figure 3, the venom (2MED) induced an increase in the size of the paw after one hour, which was 100% inhibited by the AE of *B. dichotoma* at 1:5 venom to inhibitor (w/w). *C. favosus* and *P. reticulata* inhibited by 90.5 ± 3.5 and 89.5 ± 2.7%, respectively. In the case of *P. megalophyllum*, however, inhibition reached only 35.62 ± 1.9% in the first hour, while that of *A. fragrans* achieved only 21 ± 2.1%. Da Costa et al. (2008) demonstrated that the leaves, bark, and roots of *P. radicans*, a plant used in the Amazon region for the treatment of snakebite, inhibited significantly the edematogenic activity of the venom of *B. atrox.*

Of all plant metabolites, the flavonoids and terpenoids are probably the most pharmacologically actives. The recognized properties of flavonoids include anti-inflammatory, anti-hepatoxic, and anti-hypertensive effects, as well as other functions, such as the inhibition of enzymes, including the A_2 phospholipases, which are an important component of snake venoms (Gutierrez and Lomonte, 1995). In the present study, flavonoids were identified in the aqueous extract of *B. dichotoma*, and terpenoids in those of *B. dichotoma, C. favosus* and *P. reticulata*, which suggests that these chemical compounds may be at least partly responsible for their anti-inflammatory properties regarding to the venom of *B. atrox*.

Inhibition of phospholipase activity

The PLA_2 activity of the *B. atrox* venom caused dose-dependent hemolysis in washed human red blood cells. The quantity of venom necessary to produce a halo of 10 mm was 1.25 µg/assay. A dose of 2MIHD was used in the assays that tested the inhibition of hemolytic activity.

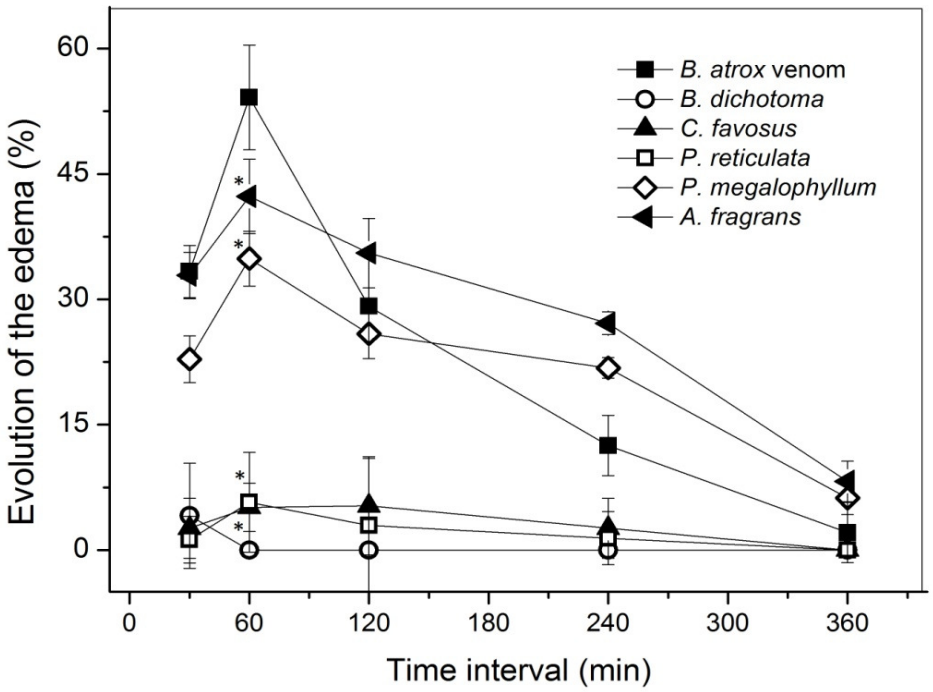

Figure 3. Inhibition of the edematogenic activity induced by the venom of *B. atrox.* Values are means± SD, n = 5.

Table 1. Phytochemical prospection of aqueous extracts of selected plant species.

Class of substance	Plant extract				
	PR (bark)	CF (bark)	PM (liana)	AF (leaf)	BD (bark)
Fatty acids	+	-	+	+	+
Anthraquinones	-	-	-	-	-
Cumarins	-	+	++	-	-
Flavonoids	-	-	-	+	+
Terpenoids	++	+	-	+	+
Condensed tannins	++	+	-	++	+
Hydrolizable tannins	++	++	+	+	++

PR = *P. reticulata*, CF = *C. favosus*, PM = *P. megalophyllum*, AF = *A. fragrans*, and BD = *B. dichotoma*. Strong reaction: ++; weak reaction: +; no reaction.

The PLA_2 activity induced by *B. atrox* venom was inhibited completely by the AEs of *B. dichotoma*, *C. favosus*, and *P. reticulata* (Table 2) at a concentration of 1:5 (w/w). The extract of *P. megalophyllum* was not effective at this concentration, however, and only produced a weak effect (19% inhibition) at 1:30, whereas the extract of *A. fragrans* reached a 50% level of inhibition at 1:30.

Aqueous extracts of *Eclipta alba*, *Mandevilla velutina*, *Miconia fallax*, *Miconia albicans*, *Stryphnodendron barbatiman*, and *Tibouchina stenocarpa* inhibited 100% of the hemolytic activity induced by the venom of *Lachesis muta* (De Paula et al., 2010). The results of the present study are nevertheless surprising due to the

Table 2. Inhibition of the phospholipase activity of *B. atrox* venom (2.5 µg/assay) by the aqueous extracts of selected plant species.

Plant species	Venom to extract ratio[a] (w/w)					
	1:1	1:2	1:5	1:10	1:20	1:30
B. atrox + *P. megalophyllum*	0.0	0.0	1.98±0.2	2.0±0.5	8.5± 0.4	19.0±0.3
B.atrox+ *A. fragrans*	0.0	0.0	0.0	17.0±0.8	20.0±0.8	50.0±0.6
B.atrox + *B. dichotoma*	33.0±0.2	54.0±0.3	100.0	100.0	100.0	100.0
B.atrox + *P. reticulata*	40.0±0.4	63.0±0.4	100.0	100.0	100.0	100.0
B. atrox + *C. favosus*	49.0±0.8	100.0	100.0	100.0	100.0	100.0

[a]inhibition of phospholipase A_2 activity (%),Values are means ± SD; n = 4.

Table 3: Inhibition of the coagulatory activity of *B. atrox* venom by the aqueous extracts of selected plant species.

Sample	Venom to extract ratio (w/w)			
	Venom	1:5	1:10	1:20
Bothrops atrox	28 s±0.01	-	-	-
B. atrox+ *P. megalophyllum*	-	33.2 s±0.02	47.5 s±0.02	1 min 8 s±0.02
B. atrox + *A. fragrans*	-	33.9 s±0.03	47.8 s±0.02	1 min 15 s±0.02
B. atrox+ *B. dichotoma*	-	6 min 42 s±0.03	7 min 9.2 s±0.03	>10 min±0.01
B. atrox+ *P. reticulata*	-	>10 min±0.01	>10 min±0.02	>10 min±0.01
B. atrox + *C. favosus*	-	>10 min±0.02	>10 min±0.02	>10 min±0.02

Values are the mean times obtained ± SD, n = 5.

relatively low concentrations at which some of the extracts were effective. In the case of *C. favosus,* for example, 100% inhibition was achieved at only a 1:2 venom to extract ratio (Table 2). Using a similar testing protocol, Magalhaes et al. (2011) recorded only 75.79% inhibition of the phospholipasic activity of the venom of Amazonian *B. atrox* at a ratio of 1:14, while Maiorano et al. (2005) recorded only 30% inhibition of the venom of *Bothrops jararacussu* by *Mikania glomerata* at a ratio of 1:200. The results of the present study thus confirm emphatically that the aqueous extracts of *B. dichotoma*, *C. favosus*, and *P. reticulata* are also a source of powerful natural inhibitors of the PLA_2 effects of viper venom.

Inhibition of coagulatory activity

The MCD of the venom of *B. atrox* used in the inhibition assays was 4.5 µg. The AEs of *C. favosus* and *P. reticulata* were capable of inhibiting completely the coagulation caused by the venom of *B. atrox* at 1:5 venom:extract, w/w (Table 3), while *B. dichotoma* at the same concentration delayed the coagulation from 28 s to 6 min 42 s. *P. megalophyllum* and *A. fragrans* were very weak inhibitors. Maiorano et al. (2005) found that the extracts of the leaves, bark, and roots of *M. glomerata* effectively inhibited the coagulatory activity of the venom of vipers (*Bothrops*) and rattle-snakes (*Crotalus*). De Paula et al. (2010) recorded 100% inhibition of the coagulatory activity of the venom of *Lachesis muta* by the aqueous extracts of *E. alba*, *S. barbatiman*, and *M. velutina.* The results of the present study demonstrated clearly that the extracts of *C. favosus* and *P. reticulata* function as potent inhibitors of the coagulatory effects of *B. atrox* venom, probably due to the inactivation of the "trombin-like" enzymes of the venom that cause the alterations to the coagulatory system.

Conclusion

The results of the present study indicate that the aqueous extracts of *B. dichotoma*, *C. favosus*, and *P. reticulata* are capable of inhibiting completely the phospholipase A_2 activity. *B. dichotoma* also inhibited by 100% the hemorrhagic and edematogenic activity provoked by the toxins present in the crude venom of *B. atrox,* while *C.*

favosus and *P. reticulata* inhibited the coagulatory effect by 100%, all results were obtained at 1:5 ratio, venom:extract (w/w). These results reinforce the potential value of traditional local knowledge for the isolation of new molecules or complementary therapies for the treatment of snakebites. While the present study has advanced the available knowledge on the principal classes of secondary metabolites and the medicinal properties of the plants extracts analyzed in relation to the crude venom of *B. atrox*. Further research is needed in order to isolate the compounds and identify their exact inhibitory mechanisms.

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