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In vivo and *in vitro* prospection of the anti-ophidic properties exercised by the extracts of *Jacaranda decurrens* L.

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ABSTRACT. The research and development of alternative treatments for snakebites (e.g., medicinal plants) is necessary due to the high costs of the existing ones. The effects of the aqueous extracts from *Jacaranda decurrens* leaves, roots, and xylopodium were analyzed upon the venom-induced (*Bothrops* spp. and *Crotalus* spp.) systemic and local toxicity. The extracts were able to partially inhibit the phospholipase activity of the venoms from *Bothrops jararacussu* and *Crotalus durissus terrificus*. The myotoxic, edema-inducing, coagulant, and hemorrhagic activities were also inhibited. The SDS-PAGE showed that the venom proteins were intact after their incubation with the extracts. This suggests that the possible mechanism of inhibition is not related to the degradation of the protein but rather to their binding to specific sites of the enzymes. The extracts significantly prolonged the survival time of animals in the lethality assay performed with *Crotalus durissus terrificus* venom and its toxin (crotoxin). The anti-ophidic activity of medicinal plants may aid in the management of snakebites in distant locations by reducing the victim's local effects and time to heal.

Keywords: snake venoms; enzyme inhibitors; medicinal plants; Bignoniaceae.

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Introduction

Snake venoms are complex mixtures of enzymes and toxic proteins, which include phospholipases A_2, myotoxins, hemorrhagic metalloproteinases, serine proteases, coagulant components, neurotoxins, cytotoxins, and cardiotoxins (Camacho et al., 2016; Xiao, Pan, Liao, Yang, & Huang, 2017; Singh, Yasir, Khare, Tripathi, & Shrivastava, 2020). Ophidian envenomations are characterized by prominent local tissue damage, i.e., hemorrhage, edema, necrosis, and alterations in the blood coagulation, as well as systemic neurotoxic effect. Additive or synergistic effects of active enzymes and toxins present in the venoms are responsible for this complex pathological condition (Sharma et al., 2016; Tasoulis & Isbister, 2017; Casewell, Jackson, Laustsen, & Sunagar, 2020).

Snakebites are considered a global problem, especially in developing countries. It is estimated that 240,000 people are bitten around the world, in which 20,000 of these cases results in death. However, it is worth mentioning that these numbers are underestimated once they are based on the notified cases in health centers. In a realistic scenario, the number of snakebites reaches 2.7 million people globally every year and 100,000 deaths (Schneider et al., 2021). Therefore, the search for venom's natural inhibitors is substantial to complement the traditional therapy, particularly regarding neutralization of local tissue damage. Plant extracts are a rich source of inhibitors and bioactive compounds that antagonize the activity of some venoms and toxins (Guimarães et al., 2014; Singh, Yasir, & Shrivastava, 2021). Only a few plant species have been studied, and the number of isolated bioactive compounds that were structurally and functionally characterized is still low (Soares et al., 2005).

Many *Jacaranda* species have been described to have pharmacological properties. In 2009 a review reported the ethnopharmacological and phytochemical characteristics of the genus *Jacaranda* (Gachet & Schühly, 2009). *Jacaranda decurrens* is a xylopodial subshrub that belongs to the Bignoniaceae family and grows in the savannah-like vegetation (cerrado *sensu stricto*) of São Paulo, Mato Grosso, Goiás, and Minas Gerais states in Brazil (Carvalho et al., 2009). Popularly known as '*carobinha*', *J. decurrens* is largely used for its anti-inflammatory, depurative, and

tonic properties found in the hydroalcoholic extract of roots. In contrast, its aerial parts are used to heal internal and external wounds (Casagrande et al., 2014). Ursolic acid, detected in this species, is a triterpene with anti-HIV-I, anti-inflammatory, antioxidant, and antitumoral properties (Shanmugam et al., 2013; López-Hortas, Pérez-Larrán, González-Muñoz, Falqué, & Domínguez, 2018; Naß & Efferth, 2021).

Therefore, a comprehensive characterization of the medicinal potential of this plant may provide a better understanding of its mechanism of action in the above-mentioned pathological processes. Thus, this study aimed to evaluate the ability of the aqueous extract (AEJd) of leaves, roots, and xylopodium from *J. decurrens* to inhibit enzymatic and pharmacological activities induced by *Bothrops* spp. and *Crotalus* spp. venoms.

Material and methods

Venoms and toxins

Venoms of *Bothrops jararacussu* (Bjussu), *B. moojeni* (Bmooj), *B. neuwiedi* (Bneuw), and *Crotalus durissus terrificus* (C.d.t) were donated by L. H. A. Pedrosa (FMRP-USP). *Bothrops jararacussu* BjussuMP-I (metalloproteinase), BjussuSP-I (serine protease), and BthTXs (myotoxins) were isolated as previously described in other studies (Andrião-Escarso et al., 2000; Mazzi et al., 2004; Sant'Ana et al., 2008).

Preparation of plant extract

Jacaranda decurrens plants were collected in Uberaba city, located in the State of Minas Gerais, Brazil. The collection site was marked using a global positioning system (GPS- Garmin legend model -79728002-19º39'58.6" S 47º59'84" W and 768 m altitude). The samples were deposited in the herbarium of the *Universidade de Ribeirão Preto* (voucher no. HPM-763).

Jacaranda decurrens leaves, roots, and xylopodium were separated, washed, homogenized using deionized water (300 g L-1) in a warring blender at room temperature, and then filtered. The aqueous extracts from both plants were centrifuged at 10,000 *g* for 10 min. The supernatants were lyophilized and stored at -20°C. Before their use, the samples were weighed, dissolved in PBS (phosphate-buffered saline) (250 μg μL-1), and stored at -2°C.

Inhibition of crude venoms and toxin activities

Crude venoms and lyophilized toxins were weighed and dissolved in PBS (50 μg μL^{-1}). In the inhibition experiments, the solutions containing a standardized amount of venom were mixed with different volumes of extract to obtain weight/weight ratios (w w^{-1}). All mixtures were incubated for 30 min. at 37°C before the evaluation of their activities.

Animals

The experiments described were approved by the Committee on Animal Research and Ethics of the *Universidade de São Paulo* (no. 08.1.202.53.1) and they were performed according to the guidelines of the Brazilian College of Animal Experimentation (COBEA).

Lethality

Doses that induced 100% lethality (LD100) in male Swiss mice (18-22 g) were determined for C.d.t and its toxin crotoxin (Biondo et al., 2003; Biondo, Soares, Bertoni, França, & Pereira, 2004). Groups of eight mice were administered with an intraperitoneal injection (i.p.) at the ratio of 1:100 (LD100 venom or toxin/AEJd, w w-1, preincubated for 30 min. at 37°C).Their survival time within 48 h was measured. PBS and AEJd were injected as controls.

Hemorrhagic activity

The minimum hemorrhagic dose (MHD) was evaluated only for *Bothrops* spp. crude venom since it produced a hemorrhagic halo of 10 mm (Biondo et al., 2003; Mazzi et al., 2004). Male Swiss mice (18-22 g) received intradermal injections in their back with all the extracts previously incubated with Bjussu, Bneuw, Bmooj, and BjussuMP-I at the ratio of 1:50 (w w^{-1}, MHD venom/AEJd). The control group received only PBS. The mice were killed three hours after the injection to measure the diameter of the hemorrhage zone in their skin.

Coagulant activity

The minimum coagulant dose (MCD) was defined as the amount of venom (Bjussu, Bmooj, Bneuw, C.d.t, BjussuSP-I) that clots 0.2 mL of plasma in 60 sec (Biondo et al., 2003; Oliveira et al., 2005). Aliquots of 0.2 mL of plasma were incubated with 50 μL of each venom, toxin, venom/AEJd, or toxin/AEJd at the ratio of 1:20 (w w^{-1}, 1 MCD venom or toxin/AEJd). The mixtures were previously incubated for 30 min. at 37°C and, afterwards, their clotting time were determined. Control included plasma incubated with PBS plus calcium or AEJd.

Phospholipase activity

The indirect hemolytic activity was evaluated in agar gels using egg yolk or erythrocytes as substrates (Biondo et al., 2003). A minimum indirect hemolytic dose (MiHD) was defined for each venom and toxin, which corresponds to the amount of enzyme that produces hemolysis zones of 10 mm in diameter. The AERJd, AELJd, and AEXJd were tested after incubation with each crude venom or toxin (Bjussu, C.d.t, or CB PLA_2) at 1:50 ratio (w w^{-1}, 1 MiHD/extract) for 30 min. at 30ºC.

Edema-inducing activity

Edema was evaluated after administration of venoms or toxin by subplantar injection in the right footpad of male Swiss mice (18-22 g). Inhibition studies were performed after preincubating venoms or toxins (Bjussu and its toxin BthTX1; C.d.t and its toxin CB PLA_2) with AELJd. Animals in the control group received an injection of PBS under the same conditions. The progression of edema was evaluated with a low-pressure pachymeter (Mitutoyo, Japan) after 10, 30, 90, and 120 min. of the injection. The dose administered of crude venoms was 0.2 μg μL^{-1} in the final volume of 50 μL. The effects of the venom or toxin/AELJd mixtures at 1:50 ratio (w w^{-1}) were evaluated (Biondo et al., 2004; Ticli et al., 2005).

Myotoxic activity

Male Swiss mice (18-22 g) received an intramuscular injection in the right gastrocnemius muscle that contained solutions of 0.4 μg μL^{-1} of Bjussu venom or BthTX-I (final volume of 50 μL per animal). The treatments evaluated were Bjussu or BthTX-I associated with AERJd, AELJd, or AEXJd (1:50 w w^{-1}). The controls were PBS or AEJd. Mice were bleeding from the tail 3 hours after the injection. The blood was collected in heparinized capillary tubes. Plasma creatine kinase activity was determined using the Kit 47-UV (Sigma Chemical Co.). The myotoxic activity was expressed in units L^{-1}, in which one unit corresponds to the production of one micromole of NADH per min at 30°C (Biondo et al., 2003).

Interaction between extracts and venoms

Sodium dodecyl sulphate - Polyacrylamide gel electrophoresis (SDS-PAGE) at a 12% concentration was used to evaluate possible protein degradation or the association with extract components. At different ratios, the venoms (Bjussu or C.d.t) were incubated with AEJd for 1h at 37ºC. The samples were then mixed with a denaturing buffer, boiled for 4 min. at 100ºC, and applied on the polyacrylamide gel for electrophoresis (Laemmli, 1970).

Proteolytic activity on fibrinogen

The methodology described by Oliveira et al. (2005) was used with some modifications. Samples of 50 μg of bovine fibrinogen were incubated with AELJd (200 μg), Bjussu (20 μg), BjussuMP-I (2 μg), Bjussu + AELJd (1:10; w w^{-1}), or BjussuMP-I + AELJd (1:10) at 37°C for 2h. The reaction was stopped by adding 25 μL of a 0.05M Tris-HCl buffer (pH 8.8) that contains 10% (v v^{-1}) glycerol, 10% (v v^{-1}) 2-mercaptoethanol, 2% (w v^{-1}) SDS, and 0.05% (w v^{-1}) bromophenol blue. Samples were analyzed by 13.5% (w v^{-1}) SDS-PAGE.

Statistical analysis

Data are presented as the mean ± SD (standard deviation) of the values obtained in all repetitions of each test. When the analysis of variance was significant, the comparison of means was performed in the Scott-Knott test ($p < 0.05$) using the R software (R Development Core Team, 2011).

Results

Survival time

The animals submitted to intraperitoneal injection of C.d.t venom or crotoxin, previously incubated with the aqueous extracts of *J. decurrens* (roots - AERJd, leaves – AELJd, and xylopodium - AEXJd) at 1:100 (w w^{-1}) ratio, showed a statistically significant increase in survival time or absence of lethality in all extracts evaluated (Table 1).

Table 1. Inhibition of the toxic activity induced by C.d.t and Crotoxin by aqueous extracts of *Jacaranda decurrens* (roots, xylopodium, and leaves).

	Survival Time (min.)			
Samples	Without AEJd[a]	AEXJd[b] (1:100 w w^{-1})	AELJd[c] (1:100 w w^{-1})	AERJd[d] (1:100 w w^{-1})
C.d.t	80.5 ± 0.5	No death*	No death*	130.5 ± 0.02*
Crotoxin[e]	105.5 ± 0.5	135.5 ± 0.5*	No death*	No death*

[a] Aqueous extracts of *Jacaranda decurrens* (AEJd), [b] xylopodium (AEXJd), [c] leaves (AELJd), and [d] roots (AERJd). [e] Enzyme (10 µg) isolated from C.d.t venom. The venom was evaluated at a dose of 30 µg. Each value represents the mean ± SD (n=8). No death = 100% of animal survival.* Significantly different from its respective positive control ($p<0.05$).

Hemorrhagic activity

The three evaluated extracts significantly inhibited the hemorrhagic activity at the 1:50 ratio (w w^{-1}). As observed, the AELJd exerted higher inhibitory activity in comparison to the other extracts, with inhibitions around 30% for Bjussu venom (20 µg), 52% for the metalloproteinase Bjussu MP-I, 42% for Bmooj venom (10 µg), and 25% for Bneuw venom (10 µg) (Figure 1).

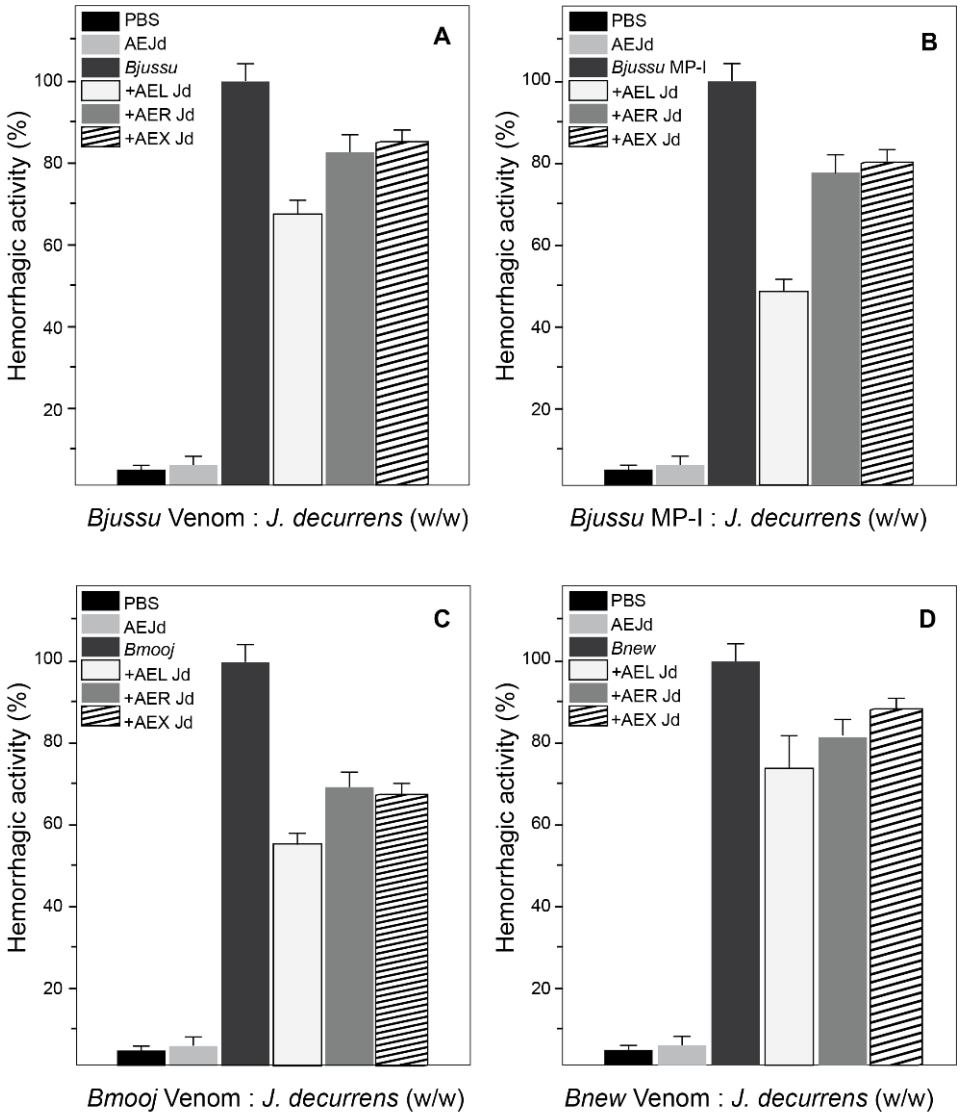

Figure 1. Inhibition of the venom-induced (*Bothrops* spp.) hemorrhagic activity by the aqueous extracts of *Jacaranda decurrens* (roots- AERJd, xylopodium- AEXJd, and leaves- AELJd). (A) Effect of *J. decurrens* extracts upon Bjussu venom. (B) Effect of *J. decurrens* extracts upon the metalloproteinase BjussuMP-I isolated from Bjussu venom. (C) Effect of *J. decurrens* extracts upon Bmooj venom. (D) Effect of *J. decurrens* extracts upon Bneuw venom. The venoms and isolated metalloproteinase were preincubated at a 1:50 (w w^{-1}) ratio for 30 min. at 37°C with the different extracts. Each experiment represents the mean ± SD (n=6). * Significantly different from the respective positive control ($p < 0.05$).

Coagulant activity

The three extracts were able to significantly increase the clotting time of citrated plasma after the addition of venoms or serine protease at a 1:20 (w w^{-1}) ratio. However, AELJd was again more efficient in inhibiting the coagulant activity of C.d.t, Bneuw, Bmooj, and BjussuMP-I (Table 2).

Table 2. Inhibition of the coagulant activity by aqueous extracts of *Jacaranda decurrens* (roots, xylopodium, and leaves).

Coagulant Activity (seconds)				
Samples	Without AEJd[a]	AEXJd[b] (1:20 w w^{-1})	AELJd[c] (1:20 w w^{-1})	AERJd[d] (1:20 w w^{-1})
PBS + Ca^{2+}	300.5 ± 0.02	-	-	-
Bjussu	47.5 ± 0.01	67.5 ± 0.02*	180.5 ± 0.02*	47.5 ± 0.01
BjussuSP-I[e]	13.0 ± 0.01	90.5 ± 0.02*	> 300.5 ± 0.01*	90.5 ± 0.02*
Bmooj	39.5 ± 0.01	45.5 ± 0.02*	> 300.5 ± 0.01*	52.5 ± 0.02*
Bneuw	42.5 ± 0.01	47.5 ± 0.02*	> 300.5 ± 0.01*	54.5 ± 0.02*
C.d.t	148.5 ± 0.01	147.5 ± 0.02	> 300.5 ± 0.01*	145.5 ± 0.02*

[a]Aqueous extracts of *Jacaranda decurrens* (AEJd), [b]xylopodium (AEXJd), [c] leaves (AELJd), and [d] roots (AERJd). [e]Thrombin-like enzyme (2 µg) isolated from Bjussu venom. The venoms were evaluated at a dose of 20 µg. Each experiment represents the mean ± SD (n = 6). * Significantly different from the respective positive control ($p < 0.05$).

Phospholipase activity

The PLA_2 activity induced by C.d.t venom and its toxin CB PLA_2 was efficiently inhibited by AELJd when previously incubated for 30 min. at 37ºC at the 1:100 (w w^{-1}) and 1:50 ratios, respectively (Figure 2). A low inhibitory activity (20%) was observed to Bjussu venom when incubated with AELJd.

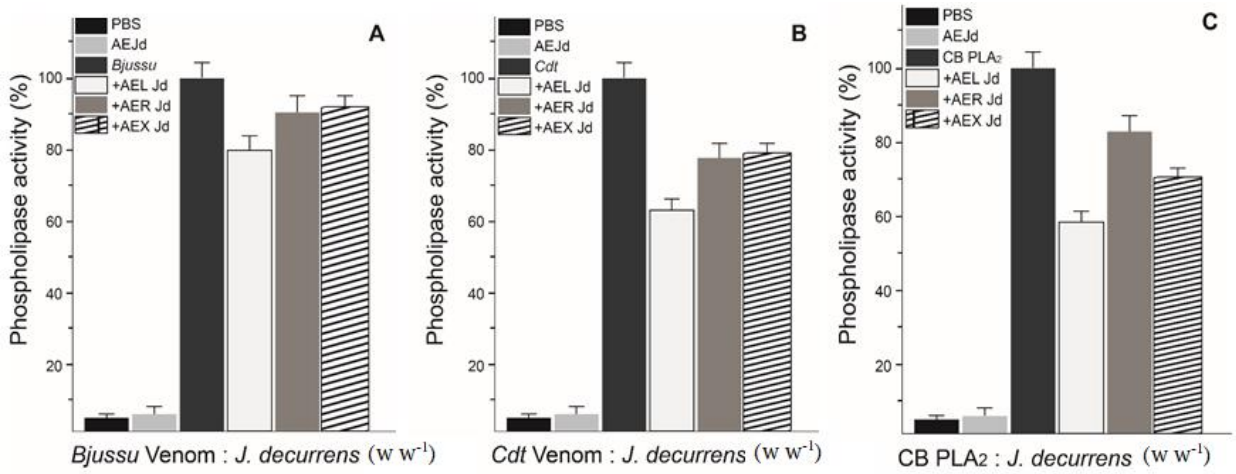

Figure 2. Inhibition of the phospholipase activity of *Bothrops* spp., *Crotalus* spp., and CB PLA_2 by the aqueous extracts of *Jacaranda decurrens* (roots-AERJd, xylopodium-AEXJd, and leaves-AELJd). Effect of *J. decurrens* extracts upon the PLA_2 activity induced by Bjussu (A) and C.d.t (B) venoms at a 1:100 (w w^{-1}, venom: extract) ratio. Effect of *J. decurrens* extracts upon the enzymatic activity induced by PLA_2 isolated from C.d.t venom (C) at a 1:50 (w w^{-1}, CB PLA_2: extract) ratio. Each experiment represents the mean ± SD (n = 3). * Significantly different from the respective positive control ($p < 0.05$).

Edema-inducing activity

The edema induced by C.d.t venom and its toxin CB PLA_2 was strongly inhibited (50 - 60%) by the leaves extract at a 1:50 (w w^{-1}) ratio (Figure 3). In contrast, the xylopodium and root extracts did not induce significant inhibition (results not shown). After 60 min. of the injection at a 1:50 ratio, the edema caused by Bjussu venom and BthTX-I toxin was inhibited (Figure 3). At a 1:100 ratio, the extracts showed an inhibitory effect within the first 30 min. after the injection (results not shown).

Myotoxic activity

The myotoxic activity induced by BthTX-I (40 µg) was strongly inhibited after incubation with the extracts at a 1:50 (w w^{-1}) ratio, which reduced the levels of creatine kinase by more than 50% when compared with the positive control (BthTX-I) (Figure 4B). However, the myotoxicity induced by Bjussu venom was less inhibited, with a higher percentage of inhibition observed for AELJd (Figure 4A).

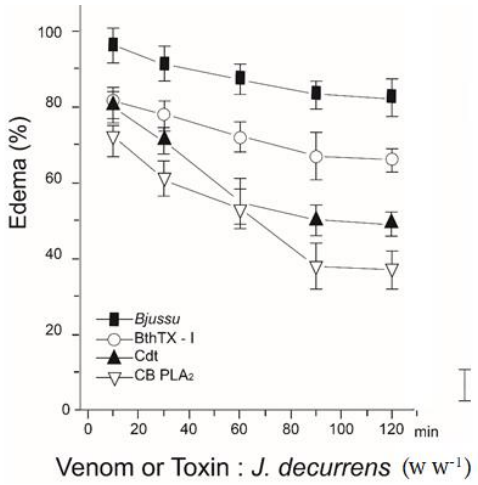

Figure 3. Inhibition of the edema-inducing activity of *Bothrops* spp. and *Crotalus* spp. venoms by the aqueous extract from *Jacaranda decurrens* leaves (AELJd). Effect of AELJd upon the edema-inducing activity of Bjussu and C.d.t venoms and their main isolated PLA_2s (BthTX-I and CB PLA_2, respectively) at a 1:50 (w w^{-1}, venom or toxin: extract) ratio. The values presented in the graphic were obtained after subtraction of venom- or toxin-induced edema. Each experiment represents the mean ± SD (n = 6).

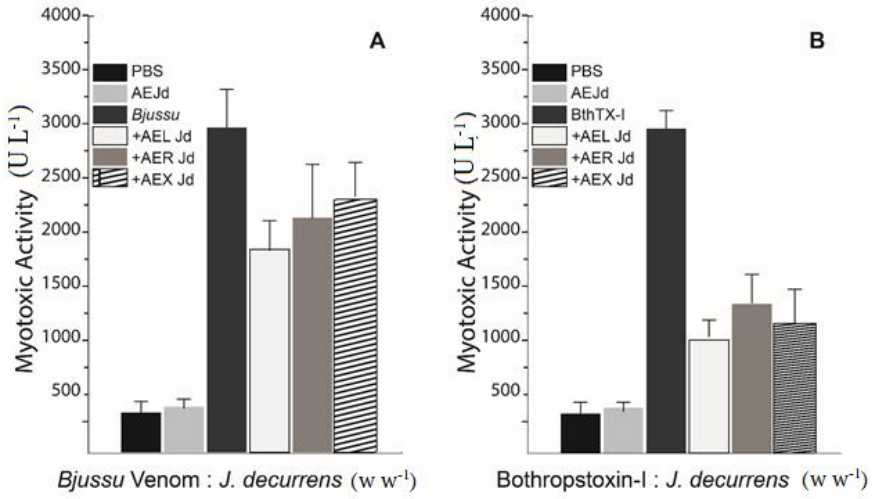

Figure 4. Inhibition of the myotoxic activity of Bjussu venom by the aqueous extract from *Jacaranda decurrens* (roots-AERJd, xylopodium- AEXJd, and leaves- AELJd). Effect of *J. decurrens* extracts upon the myotoxicity induced by Bjussu (A) and its main isolated myotoxic PLA_2 (BthTX-I) (B) at a 1:50 (w w^{-1}, venom or toxin: extract) ratio. Each experiment represents the mean ± SD (n = 6).* Significantly different from the respective positive control ($p < 0.05$).

Interaction with proteins and inhibition of proteolysis

An SDS-PAGE was performed to understand better the mechanisms related to the inhibitory action of the AEJd extracts upon snake venoms and toxins. The extracts did not exercise any proteolytic effect nor interfere with the solubility of the venom/toxin (Figure 5A). On the other hand, it was observed that the most active extract, AELJd, significantly reduced the venom-induced proteolysis of fibrinogen. This fact is evidenced by the preservation of the α and β chains in the fibrinogen molecules submitted to the venoms (Figure 5B).

Discussion

Snakebite is a neglected medical condition, affecting predominantly rural areas far from health centers (Williams et al., 2017; Martins et al., 2019; Schneider et al., 2021). Although serum therapy is the most recommended treatment for snakebites, this protocol is expensive and not available for everyone. Also, the high biodiversity of snakes makes the serum be sometimes very unspecific and inefficient (Gutiérrez, Williams, Fan, & Warrell, 2010; Williams et al., 2017). Throughout human history, several communities discovered and applied natural treatments to many ailments (Yuan, Ma, Ye, & Piao, 2016; Kim, Kismali, & Gupta, 2018; Elkordy, Haj-Ahmad, Awaad, & Zaki, 2021). Besides having a low cost, plant extracts and

phytochemicals are considered efficient in many clinical trials and laboratory assays. In this study, we present for the first time the anti-ophidic properties of *J. decurrens* aqueous extracts.

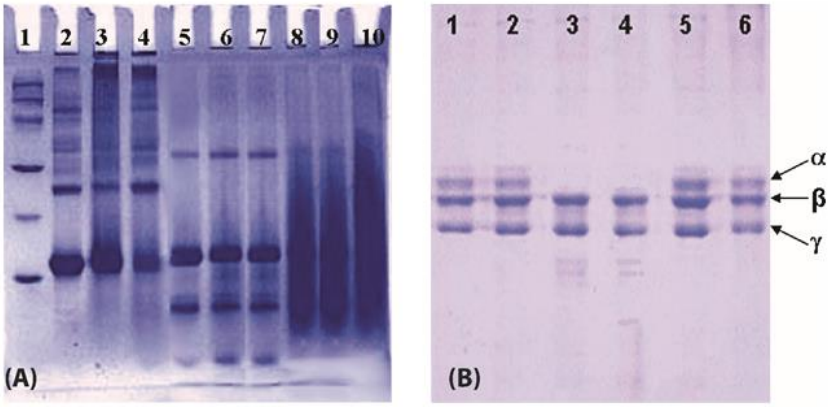

Figure 5. Eletrophoresis analysis. (A): SDS-PAGE for visualization of the interaction between snake venoms and *Jacaranda decurrens* extracts, after incubation for 30 min. at 37°C. Samples: 1- Bjussu venom (20 µg); 2- Bjussu (20 µg) + Aqueous extracts of leaves (AELJd) (1:50, w w^{-1}); 3- Bjussu (20 µg) + Aqueous extracts of roots (AERJd) or Aqueous extracts of xylopodium (AEXJd) (1:50, w w^{-1}); 4- C.d.t (20 µg); 5- C.d.t (20 µg) + AELJd (1:50, w w^{-1}); 6- C.d.t (20 µg) + AERJd or AEXJd (1:50, w w^{-1}); 7- AELJd (1000 µg); 8- AEXJd (1000 µg); 9- AERJd (1000 µg). (B): SDS-PAGE for visualization of the inhibitory effect of *J. decurrens* extracts upon the fibrinogenolytic activity of Bjussu venom. Samples: 1- Fibrinogen (75 µg); 2- Fibrinogen + AELJd (200 µg); 3- Fibrinogen + Bjussu (20 µg); 4- Fibrinogen + BjussuMP-I (2 µg) 5- Fibrinogen + Bjussu + AELJd (1:10, w w^{-1}); 6- Fibrinogen + BjussuMP-I + AELJd (1:10, w w^{-1}).

The results demonstrated that the aqueous extracts obtained from *J. decurrens* leaves, xylopodium, and roots possess inhibitory effects against some activities induced by snake venoms and toxins, both *in vitro* and *in vivo*. One of the best results obtained in the present work is prolonging survival time after administering snake venom/toxin preincubated with different AEJd (Table 1). Previous works have shown that plant extracts, such as *Euphorbia hirta* and *Cassia auriculata,* improved survivability in animal models (Gopi, Renu, Vishwanath, & Jayaraman, 2015; Urs et al., 2015). Scientific results in this context are relevant since the extracts can give sufficient time to take the victim to a health center to receive adequate treatment. Hemorrhage is a severe symptom that occurs in some accidents with *Bothrops* snakes. Here we demonstrated that the extracts used could significantly reduce the action of proteases, especially metalloproteinases, which are mainly responsible for the hemorrhagic effect (Figure 1). Similar effects were described by Mourão et al. (2014), which achieved a drastic reduction in the hemorrhagic effect induced by *B. jararaca* with the hydroalcoholic extract from *Mikania glomerata.*

Chemical prospection studies confirmed the presence of flavonoids, saponins, steroids, triterpenes, starch, coumarins, resins, tannins and anthraquinones (Zatta et al., 2009). According to Moura et al. (2016), tannins can form complexes with calcium, which is a cofactor of phospholipases A_2 and various enzymes involved in the coagulation cascade. On the other hand, flavonoids have the ability to bind to the amide groups of different proteins by strong hydrogen bonds (Mors, Nascimento, Pereira, & Pereira, 2000).

Snake venom PLA_2 is a multifunctional enzyme involved in myotoxicity, edema induction, membrane disruption, induction of apoptosis, inflammation, and neurotoxicity. *J. decurrens* leaves extract induced significant inhibitions (around 40%) on the PLA_2 activity (Figure 2). Furthermore, the myotoxicity of the PLA_2 from BthTX-I was efficiently inhibited by all AEJd (Figure 4). Many plant extracts were described as PLA_2 inhibitors (Félix-Silva et al., 2014; Alam et al., 2016; Marques et al., 2021). Phospholipases A_2 promotes inflammatory reactions by breaking down membrane phospholipids releasing arachidonic acid and lysophospholipids, which result, among other effects, in edema. Preincubation with AELJd significantly reduced the accumulation of inflammatory fluid on mice paws by 50 to 60% (Figure 3).

Another significant result obtained in the present work was the increase in the clotting time induced by snake venoms and the serine proteinase BjussuSP-I (Table 2). Coagulopathies resulting from snakebites correspond to complications that can lead to hemorrhage, heart attack, systemic shock, and thrombosis. Although it seems controversial (increase in clotting time and anti-hemorrhagic effect), this effect is due to the inhibitory nature of the extract towards different enzymes present in the venoms rather than promoting some activity. The nature of this inhibition is still unknown, although some mechanisms have been proposed. We performed an SDS-PAGE to visualize possible interactions between enzymes/extracts, but no proteolysis

or alterations in the migration profile of toxins were observed (Figure 5A). The most probable mechanism exerted by the compounds of AEJd is the ability to bind to specific sites on the structure of toxins, reducing their catalytic activity and altering, consequently, other related activities. In summary, further analysis using specific fractions of the extract, or its major components, are still needed since these findings may lead to more information about the inhibitory mechanisms.

Although many studies have described a large number of plants that present inhibitory activities towards snake venoms and toxins, the endemism and seasonality of some species make it necessary to investigate more species with anti-ophidic properties. Data obtained in this work, although significant, does not replace serum therapy but rather suggests a complement and a first aid in cases where the victim is in a hard-to-access geographical location. In addition, well-conducted studies using species at risk or in degraded environments could demonstrate their medical value and protect them from extinction.

Conclusion

Jacaranda decurrens extracts were evaluated upon the lethality, hemorrhage, phospholipase, and fibrinogenolytic activities, but also in assays evaluating edema, myotoxicity, and human plasma coagulation. They exerted significant inhibition on different snake venoms and isolated toxins (phospholipases A_2, serine proteases, and metalloproteases). Considering the role of proteases and phospholipases A_2 in processes related to inflammation and hemostasis, the modulating action observed in the present work is essential as a basis for future studies that may lead to the development of new therapies.

However, new studies are needed to identify the molecules responsible for the effects observed in each extract. Also, it is necessary to elucidate the active inhibition mechanisms, which will enable future applications of the extracts.

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