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Alexiteric potential of *Croton urucurana* Baill. against *Bothrops diporus* venom

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Abstract

Croton urucurana Baill., traditionally used in South American medicine, is explored for its alexiteric potential against *B. diporus* venom, addressing limitations of antivenom therapy in preventing local tissue damage. This study evaluates the antivenom properties of *C. urucurana* extracts, essential oils, and latex against *B. diporus* venom using *in vitro* and *in vivo* models, focusing on phytochemical profiles and seasonal variations. Plant materials (leaves, bark, latex) from spring and summer were extracted using polar and non-polar solvents. Essential oils were analysed by GC-MS, and phytochemicals (phenolics, flavonoids, tannins) were quantified. Alexiteric activity was assessed through SDS-PAGE, proteolytic, coagulant, and haemolytic inhibition assays, and an *in vivo* dermonecrosis model using a 3% latex preparation. Latex collected in summer exhibited the highest alexiteric potential, effectively inhibiting venom-induced proteolysis, coagulation, and haemolysis. Topical application of latex reduced dermonecrosis, inflammation, and extracellular matrix degradation in mice. Polar extracts (methanolic, ethanolic) showed moderate activity, with proanthocyanidins tentatively identified as key bioactive via acid-butanolysis and HPLC-DAD analysis. Comparison between spring and summer collections showed consistent latex efficacy, suggesting functional stability across these evaluated seasons. *C. urucurana* latex is a promising phytotherapeutic agent for mitigating *B. diporus* venom-induced tissue damage, supporting its traditional use and warranting further chemical and clinical investigations.

KEYWORDS
dermonecrosis, Dragon's blood, proanthocyanidins, snakebite, sPLA2

Impact statement

Snakebite is an important health problem in South America, particularly because antivenom treatment does not always prevent the local tissue damage caused by venom. This work is important because it addresses the need for complementary approaches to reduce these harmful effects, focusing on Bothrops diporus, an important venomous snake

in northeastern Argentina. The study advances the field by providing a comprehensive evaluation of Croton urucurana preparations against the damaging effects of this venom and by identifying the latex as the most effective preparation. Importantly, the work provides new evidence that topical application of C. urucurana latex can reduce tissue destruction and inflammation and preserve skin structure following venom exposure. These findings strengthen the scientific basis for the traditional use of this plant and provide a potential starting point for developing complementary treatments aimed at limiting local tissue damage after snakebite.

Introduction

Snakebite envenomation is a high-priority Neglected Tropical Disease designated by the World Health Organization (WHO), affecting millions of people worldwide and causing over 100,000 deaths annually alongside severe long-term tissue damage and disability [1]. In Argentina, Paraguay, the south-central portion of Brazil, and Bolivia, envenomations represent a major public health concern, with *Bothrops diporus* being particularly prevalent in northeastern Argentina, where it accounts for a significant proportion of snakebite cases [2]. Envenomation by this species typically causes severe local symptoms, swelling, haemorrhage, blistering, pain, compartment syndrome, and necrosis, mainly due to the action of group II secreted phospholipase A_2 ($sPLA_2$-II), thrombin-like enzymes, and snake venom metalloproteinases (SVMPs) [3, 4]. $sPLA_2$-II promote myonecrosis by disrupting skeletal muscle cell membranes and intensifying oedema and inflammation, whereas SVMPs degrade basement membrane and extracellular matrix components, compromising microvascular integrity and causing local haemorrhage. Thrombin-like enzymes further disturb local haemostasis through fibrinogen cleavage, thereby exacerbating bleeding and tissue injury. Collectively, these toxins damage muscular, connective, and endothelial tissues and trigger inflammatory responses through tissue injury and immune cell activation [4–6].

Previous *in vitro* studies have characterised the venom's procoagulant effects on human plasma, demonstrating rapid clotting via activation of coagulation factors, with kinetic modelling highlighting the role of serine proteases [7]. Additionally, *in vivo* animal studies in mice have established the venom's lethality profile, using intraperitoneal (I.P.) administration, with LD50 values ranging from 51.8 to 82.6 μg/mouse across Argentine specimens, alongside consistent enzymatic activities inducing hemorrhage, myotoxicity, and dermonecrosis [2, 3]. Cytotoxicity assays on mammalian cell lines further confirm venom-induced cell death and morphological changes, underscoring its potent local tissue-damaging effects [8]. These findings justify the use of *B. diporus* venom in animal models to evaluate potential therapeutics against its envenomation effects, as the venom's pathological actions *in vivo* closely mimic human clinical presentations.

Currently, the only WHO-approved treatment for snakebite envenomation is immediate antivenom therapy [1]. However, while antivenom effectively neutralises circulating toxins to prevent systemic lethality, it exhibits limited efficacy in reversing localised tissue damage or arresting secondary inflammatory cascades that have already been initiated prior to administration [9]. This challenge underscores the need for complementary therapeutic strategies. Ethnopharmacology presents promising alternatives, with numerous studies supporting the efficacy of medicinal plants in alleviating local symptoms and reducing tissue damage following snakebite envenomation [10, 11].

Croton urucurana Baill. (Euphorbiaceae), commonly known as "*Sangre de Drago*" (Dragon´s blood), is a South American native plant traditionally used for a wide range of ailments [12, 13]. Different parts of the plant, including bark extracts and latex (sap), have been reported to exhibit analgesic [14], gastroprotective [15], anti-edematogenic [16], antimicrobial [17], wound-healing [18], hypolipidemic, renoprotective, and cardioprotective properties [19]. Furthermore, aqueous leaf extracts can inhibit the haemorrhagic activity of *Bothrops jararaca* venom, with proanthocyanidins identified as key compounds reducing venom-induced haemorrhage by up to 70% in enzymatic assays [20]. *In vivo* studies in mice have also demonstrated that aqueous extracts of *C. urucurana* mitigate oedema and myonecrosis induced by *Bothrops atrox* venom, prolonging survival time and reducing local tissue damage [21]. Furthermore, the stems, bark and leaves essential oils contain anticancer bioactive agents [22–24]. The latex, known as Dragon's blood, is applied both topically for wound healing, ulcers, and local tissue repair, and orally for gastrointestinal ailments such as dysentery [25]. It has also been reported to exert analgesic, antibacterial [15], antifungal [26] and antitumor properties [27].

These prior *in vitro* and *in vivo* findings on the anti-haemorrhagic and tissue-protective effects of *C. urucurana* against related Bothrops venoms provide a scientific basis for evaluating its alexiteric potential, the capacity of non-immunological-based agents to directly neutralise or inhibit snake venom components [28, 29], using an animal model of *B. diporus* envenomation.

Despite its documented pharmacological potential, there is a lack of scientific evidence regarding the specific efficacy of *C. urucurana* extracts and latex against *B. diporus* venom local tissue-damaging effects. In this study, we chemically characterise and evaluate the alexiteric potential (using *in vitro* and *in vivo* models) of extracts from different plant organs, considering seasonal variation and solvent polarity.

Materials and methods

Plant material

Samples of plant material were collected from stable populations of *C. urucurana* using pruning-like methods to ensure the preservation of this native species. *C. urucurana* is classified as Least Concern (LC) according to IUCN Red List criteria and is not listed as a threatened or protected species under Argentine national or provincial regulations. *C. urucurana* populations were from Corrientes, Argentina, located along the Paraná river (27°22′15.5″ S, 58°41′34.9″ W). Specimens were taxonomically identified by Dr.

Walter Medina, and a voucher was deposited at the Herbarium of the Instituto de Botánica del Nordeste as *C. urucurana* Baill. (Torres, A.M. 29, CTES0072631) following the systematics of Chiarini F. [30]. Collection was conducted on public land, with permission obtained from the Dirección de Recursos Naturales de la Provincia de Corrientes, in accordance with regulation number 845 of scientific collection and National laws. No endangered species were impacted, and sampling complied with institutional and national guidelines for native plant collection.

Samples were collected during two distinct phenological stages in 2022: summer (Stage III, February 2022) and spring (Stage II, October 2022). According to records from the Argentinian Meteorologic Service[1], the summer sampling period in Corrientes experienced mean temperatures of 27.5 °C (maximum highs >35.0 °C) and total monthly precipitation of 35 mm. In contrast, the spring sampling (Stage II) period recorded a mean temperature of 23.2 °C and total monthly rainfall of 85 mm. Latex (Ltx) was obtained by incisions in the trunks of adult specimens (n = 3) and by collecting the exudate. In addition, leaves (L) and bark (B) were harvested separately for organ-specific bioprospecting. All plant material was air-dried at room temperature (25 ± 2 °C) under ambient laboratory conditions (relative humidity 40–60%) for 3 days.

Experimental animals

Young male BALB/c mice (18–20 g and three-week-old) were obtained from the animal facility of the Faculty of Medicine, Universidad Nacional del Nordeste (UNNE), Argentina. Animals were housed under standard laboratory conditions (ambient temperature: 25.0 ± 2.0 °C; relative humidity: 55–65%; 12 h light/dark cycle) with free access to food and water. All procedures involving animals were conducted in strict accordance with international ethical guidelines for the care and use of laboratory animals. Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the School of Medicine, UNNE (Res. No. 0013 CICUAL-Med-UNNE/22). Only male mice were used, in accordance with previously established experimental protocols [31], to maintain consistency with previous studies and minimise biological variability associated with sex-related differences that could influence the experimental outcomes.

Bothrops diporus venom

Venom samples were obtained by standard milking procedures from 12 adult female specimens of *B. diporus*, from Corrientes (Capital Department, Argentina). Only adult snakes were included, with adult status determined using morphometric parameters such as snout-to-vent length of 50.9 cm corresponding to a minimum age of 24 months [32]. The captured and/or rescued snakes were in captivity at the Argentine Interactive Centre for Venomous Snakes (CISVA) of the Faculty of Veterinary Medicine at UNNE. They were identified and handled by trained personnel authorized by Resolution No. 1473/25 (Dirección de Recursos Naturales de la Provincia de Corrientes). CISVA has a secure animal facility with controlled conditions (24–26 °C, 12-h light/dark cycle, with water and prey), and the snakes were hand-milked using non-invasive techniques to minimise stress. The handling and extraction of the venom were carried out specifically by Dr Pamela Teibler, Director of CISVA. Animals were not purchased or privately owned. The venom was dried under reduced pressure and stored at −20 °C until use. Before use, it was reconstituted in a physiological saline solution. Venom concentrations were expressed as dry weight.

Extracts preparation

Ground leaves (L) and bark (B) (20 mesh) were subjected to maceration for 48 h at a 1:10 (w/v) solid-to-solvent ratio under continuous orbital agitation (100 rpm) at room temperature. The extraction process was performed in one step for each plant sample. Based on previous results no significant yields were obtained with subsequent extractions when working with 1:10 solid-to-solvent ratio. The extracts were filtered and dried on a rotary evaporator under vacuum until a solid residue remained. The yield of each extract was calculated. The aqueous extracts were lyophilised (−58 °C, 0.035 mBar). The latex (Ltx) was dried using a rotary evaporator. All extracts were stored in a vacuum desiccator until utilisation and reconstituted with the corresponding solvent before analysis. Data are expressed in terms of dry weight.

Extraction and characterisation of essential oils

Extraction

Essential oils (Eos) were obtained from leaves (Eo-L) and bark (Eo-B) of *C. urucurana* by hydro-distillation, involving the direct passage of water vapour through the plant material for 90 min. After complete condensation, the volatile compounds were collected using a Florentine flask. Yield was determined, and the oils were stored at −20 °C in amber vials under N_2 atmosphere.

GC-MS analysis

Chemical analyses were performed in duplicate using a Shimadzu GC2010 coupled to a Shimadzu QP2020 Ultra Mass Spectrometer (Shimadzu Corporation, Kyoto, Japan) in total ion current mode (40–400 m/z). A 30 m × 0.25 mm i.d. × 0.25 μm HP-5MS column (95% dimethyl-5% diphenylpolysiloxane; Agilent Technologies, Wilmington, DE, USA) was used. The oven temperature program was: 60 °C (8 min), increase to 180 °C at 3 °C/min, then to 250 °C at 20 °C/min, held for 10 min. Helium (1.0 mL/min) was the carrier gas. Samples (0.2 μL) were injected in split mode (1:55). Injector, interface, and ion source temperatures were 250 °C; ionisation voltage was 70 eV and acquisition mass range 40–400 a.m.u.

The volatile organic compounds were tentatively identified by comparing the mass spectra of the samples with the data system [33] and based on arithmetic retention indices calculated using a series of n-alkanes (C9–C26) and comparing with either reference substances or literature values. Whenever it was possible, MS identification was confirmed with authentic references. The relative amounts of

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components present in the essential oil were expressed as percentages of the peak area of total chromatograms, without corrections for response factor.

Phytochemistry

Total phenolic content (TPC)

The total phenolic content in the samples was evaluated using the Folin-Ciocalteu reagent [34]. Absorbance was measured at 750 nm using a microplate reader (Thermo MultiSky Scan). Determinations were performed in quadruplicate, and the results were expressed as μg of gallic acid equivalents per mg of dry extract.

Total flavonoid content (TFC)

Total flavonoid content was determined using the spectrophotometric method based on $NaNO_2$ and $AlCl_3$ [35]. The extracts (100 μL) were diluted and mixed with 10 μL of 5% $NaNO_2$. After 5 min, 10 μL of 2% $AlCl_3$ were added and left reacting for 6 min. Finally, 60 μL of 4% NaOH were added, thoroughly mixed, and absorbance was read at 500 nm using a microplate reader. Results were expressed as μg catechin equivalent (CE)/mg dry extract.

Total condensed tannin (TCT)

Total condensed tannin content was determined using the vanillin-HCl spectrophotometric method described by Herald et al. [36]. In a 96-well microplate, 30 μL of each extract were mixed with 150 μL of vanillin reagent and incubated for 20 min at 30 °C, and the absorbance was read at 500 nm. A calibration curve was made using catechin as standard, and results were expressed as μg catechin equivalent (CE)/mg dry extract. Determinations were performed in quadruplicate.

Proanthocyanidin study

Latex was submitted to butanolysis following the methodology proposed by Porter et al. [37] with slight modifications. Briefly, 2 mg of latex (L-III) were dissolved in 1 mL methanol and placed in an 8 mL capacity thick-walled, screw-top glass tube sealed with a Teflon-lined screw cap. To each tube, 6 mL of a solution of n-butanol (n-ButOH): 37% (w/v) HCl (95:5) and 200 μL of a 2% (w/v) of $NH_4Fe(SO_4)_2.6H_2O$ in 2 M HCl were added. The tubes were capped, thoroughly mixed and heated at 90 ± 0.5 °C for 40 min. The solutions were cooled and further analysed by UV-visible spectrophotometer and HPLC-DAD.

HPLC-DAD analysis was performed using a Shimadzu LC-20AT HPLC (Tokyo, Japan) following the method previously described Pan et al. [38]. The mobile phase was composed of 3% (w/v) formic acid pH = 2.2 (Phase A) and methanol (Phase B). Injection volume of 20 μL. The flow rate was 0.7 mL min^{-1} and the elution gradient profile as follows: 0–5 min 20% of B; 5–10 min 35% of B; 10–20 min 50%, 20–30 min 70% B, 30–50 min 70% of B, followed by column washing and reconditioning (10 min). A Hypersil ODS column 250 × 4.6 mm, 5 μm particle size (Thermo Scientific, Whatman, MA, USA) was used and the UV–Visible DAD detector Shimadzu, SPD-20 MA (Tokyo, Japan) was set at 254, 280 and 520 nm.

Screening of alexiteric activity

The protein composition of *B.diporus* venom was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using a Mini-Protean IV system (Bio-Rad). Protein separation was performed on a 12% resolving gel with a 4% stacking gel, following the protocol described by Laemmli [39]. Gels were stained with Coomassie Brilliant Blue R-250 for protein visualisation. To assess the alexiteric potential of the samples (extracts/Ltx/Eo), 1.8 mg of plant extract was weighed, dissolved in 15 μL of solvent, and mixed with 10 μL of venom solution (1 mg V in 40 μL of SF). The venom:extract mixture (1:10) was then incubated for 30 min at 37 °C. After incubation, 25 μL of sample buffer and 2.5 μL of mercaptoethanol were added, and the mixture was denatured at 100 °C for 5 min. Finally, the samples were centrifuged prior to loading (2000 x g for 5 min) to remove insoluble material, and 3.5 μL of the supernatant was loaded for SDS-PAGE analysis.

Changes in the banding pattern compared to the untreated venom lane were interpreted as indicative of potential antivenom activity [40]. Solvent controls were performed to assess any potential solvent-related effects, as well as controls using the extract in the absence of venom to determine whether plant constituents might interfere with the interpretation of the results.

Neutralisation of proteolytic activity

The ability of *C. urucurana* samples (extracts/Ltx/Eo) to neutralise the proteolytic activity of *B. diporus* venom was assessed using a modified SDS-PAGE protocol as described by Gay et al. [41]. A standard representing complete proteolysis was prepared by incubating venom (1 mg/mL) with casein (10 mg/mL in 100 mM Tris-HCl buffer, pH 8.0) at a 1:10 mass-to-volume ratio for 60 min at 37 °C. To evaluate inhibition of enzymatic activity, venom was pre-incubated with each sample at a 1:30 (venom:sample) ratio for 60 min at 37 °C. Following pre-incubation, casein (10 mg/mL) was added, and the mixture was incubated for an additional 60 min at 37 °C. Control reactions were run in parallel by incubating the plant samples with casein alone to rule out nonspecific effects. In addition, solvent controls corresponding to the solvents used for extract reconstitution were included in all assays. All samples and controls were analysed by SDS-PAGE. To evaluate anti-proteolytic activity semi-quantitatively, densitometric analysis of gel images (300 dpi) was performed using ImageJ software [42]. Neutralisation was considered positive (+) when the densitometric value of the evaluated lane remained above 50% of the value obtained for the intact casein lane.

Neutralisation of coagulant activity

The minimum coagulant dose (MCD) was defined as the amount of *B. diporus* venom required to clot 100 μL of citrated plasma (ratio 1:9; plasma:sodium citrate 3.8%) within 60 s. In this assay, the MCD corresponded to 4 μg, which is equivalent to 100 μL of a venom solution (40 μg/mL). Coagulant activity was measured

using a semi-automated coagulometer (Col 1 – Wiener Lab, Argentina). To assess neutralisation, venom was pre-incubated with each sample at a 1:20 (venom:sample) ratio for 30 min at 37 °C. The degree of inhibition was expressed as % Inhibition of coagulant activity (%InhC) = [(SCT - MCD) x 100]/[(500 – MCD)], where SCT: sample coagulation time. This calculation is based on the differences between the venom-induced clotting time (MCD) and the clotting time of the venom pre-incubated with extract (SCT), the 100% inhibition reference is set as 500 s which corresponds to a tenfold increase in clotting time relative to that obtained for the MCD [43]. Controls involving the solvents used for the reconstitution of the extracts were also assayed. All assays were performed in triplicate.

Neutralisation of indirect haemolytic activity

The capacity of *C. urucurana* samples to inhibit the haemolytic activity of phospholipase A_2 (PLA_2) from whole *B. diporus* venom was evaluated using an indirect haemolysis assay on agarose - human erythrocyte - egg yolk gel plates. The minimum indirect haemolytic dose (MIHD) was defined as the amount of venom that produced a haemolytic halo 10 mm in diameter after 20 h of incubation at 37 °C [40]. To assess neutralisation, venom at MIHD was pre-incubated with each sample at a 1:20 ratio for 30 min at 37 °C. The venom–sample mixtures were then applied to the gel plates and incubated for 20 h at 37 °C. Controls involving the solvents used for the reconstitution of the extracts were also assayed. All determinations were performed in quadruplicate. Results were expressed as percentage inhibition (%Inh) relative to the halo diameter induced by the MIHD alone.

Inhibitory effect of *C. urucurana* latex on *B. diporus*-induced dermonecrosis

A Lanette-based cream containing 3% *C. urucurana* latex was formulated and to evaluate its potential to inhibit local effects of *B. diporus* venom. The cream was prepared in the laboratory of Veterinary Faculty of UNNE, using 10 g of Lanette SX base cream, with the addition of 4 g of solid vaseline and 9 g of cetyl and stearyl alcohol, 74 g of distilled water, mixed with 3% (w/w) lyophilized latex in a sterile mortar under aseptic conditions to ensure homogeneity and stability. A dermonecrotic dose of venom (1.5 mg/mL in PBS; injection volume: 100 µL) was used, defined as the amount that produces a necrotic lesion larger than 5 mm in diameter (using a digital calliper 0.01 mm precision) within 48 h without causing death in mice [44]. Four experimental groups were established (n = 5 animals per group): (1) a negative control group receiving PBS only; (2) a cream-only control group; (3) a positive control group receiving intradermal injection of venom into the shaved dorsal skin of anesthetized mice (chloral hydrate, 50 mg/kg, intraperitoneal injection for sedation to minimize pain and distress during the procedure); and (4) a treatment group in which mice received venom followed by topical application of the latex cream 15 min later. Cream was reapplied every 12 h for three consecutive days.

Macroscopic evaluation of dermonecrosis was conducted at defined time intervals post-injection. At the end of the experiment, animals were euthanised by cervical dislocation following ethical guidelines. Tissue samples from the lesion site were collected, fixed in 10% buffered formalin (w/v) for 24 h, and processed using standard histological techniques. Staining was performed using hematoxylin and eosin (H&E) and Gomori's trichrome methods. Histopathological changes were documented photographically using a Zeiss Axiovert 5 binocular microscope equipped with a 10 MP AxioCam 208 colour camera.

Histopathological changes were semi-quantitatively evaluated by two independent observers blinded to treatment groups using a score ranging from 0 to 3: 0 (absent); 1 (mild); 2 (moderate); 3 (severe), for three criteria: epidermal damage, inflammatory cell infiltration and extracellular matrix degradation. The maximum total score per tissue section was 9. Data were expressed as mean ± SD for each experimental group.

Statistical analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical analyses of quantitative phytochemical assays and *in vitro*/*in vivo* neutralization evaluations were conducted using GraphPad Prism® version 8.0 (GraphPad Software, San Diego, CA, USA). Differences among experimental groups were analysed by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons, with statistical significance defined at $p < 0.05$. Pearson correlation and multivariate hierarchical cluster analysis was performed using InfoStat software version 2015 (Córdoba, Argentina), variables were standardised before analysis, Euclidean distance matrices and the average linkage agglomerative method were used to evaluate relationships among plant organs, extraction solvents, and seasonal collections.

Ethics approval and consent to participate

All animal experiments were approved by the IACUC of the Faculty of Medicine, UNNE (Res. No. 0013 CICUAL-Med-UNNE/22). Plant collection permissions were obtained from the Dirección de Recursos Naturales de la Provincia de Corrientes. The handling of snakes was carried out in accordance with National Law No. 22,421, with permission from the Dirección de Recursos Naturales de la Provincia de Corrientes (Provision 1473/25). No human participants were directly involved in this study. Red blood cells were obtained from surplus material of routine clinical samples collected on the same day at the Faculty Clinical Analysis Laboratory (Laboratory of Applied Biochemistry), prior informed consent from the subjects.

Results and discussion

Yields of extracts and essential oils from *C. urucurana*

The yields of essential oils and extracts from *C. urucurana* obtained across two seasons (summer and spring) from the various plant organs studied are presented in Table 1. Seasonal yield variations in *C. urucurana* reflect shifts between growth and defense metabolism. In spring (Stage II), resources favour primary

TABLE 1 Yields of extracts and essential oils from different organs of *C. urucurana*.

Yield (%)	Eo- L	Eo-B	L1	L2	L3	L4	B1	B2	B3	B4
Spring (II)	0.10	-	9.00	7.00	2.00	9.00	5.00	3.00	0.40	8.00
Summer (III)	0.06	0.01	11.00	12.00	4.00	2.00	5.00	6.00	0.70	5.00

Eo-L leaves essential oils, Eo-B bark essential oils. Leaves extracts: L1: methanolic, L2: ethanolic, L3: hexanic, L4: aqueous. Bark extracts: B1: methanolic, B2: ethanolic, B3: hexanic, B4: aqueous. Results are expressed as percentages (g of extract/essential oils by 100 g of dry plant material).

growth, cell expansion, and tissue hydration, enhancing polar constituent solubility. In summer (Stage III), thermal, light, and hydric stress prompt a shift toward secondary metabolism, increasing protective phytometabolites (polyphenols, condensed tannins, and volatile terpenoids). Additionally, summer cell wall lignification and tissue maturation alter solvent penetration, further accounting for the observed yield differences [12, 13, 22].

The yield of latex (Ltx) was not expressed as a dry weight extraction percentage of plant material because the exudate was collected directly from trunk incisions. Overall, a total crude latex mass of 2.5 g was obtained from a total of 3 adult trees, specimens with a girth at breast height (GBH) of at least 90 cm. In summer, the extracts with the highest yields were the methanolic, ethanolic, and aqueous leaf extracts (L1, L2, and L4, respectively), while in spring highest yields were obtained for the methanolic and aqueous leaf extracts (L1 and L4), followed by the aqueous bark extract (B4). The variations observed could be a consequence of the relative changes in compound contents and composition due to seasonal variations, as well as the effectiveness of each solvent in extracting different groups of compounds [45].

The essential oil yield was higher during spring when obtained from the leaves and minimal or undetectable when obtained from the bark for both seasons. Characterising the essential oil profile of the bark is of interest given the close anatomical proximity of cortical secretory tissues and laticiferous canals in the trunk bark of *Croton* species [23, 46]. While a potential contribution or co-extraction of lipophilic volatile constituents during latex tapping remains hypothetical, profiling the bark essential oil provides useful comparative insight into the overall volatile metabolome of *C. urucurana*.

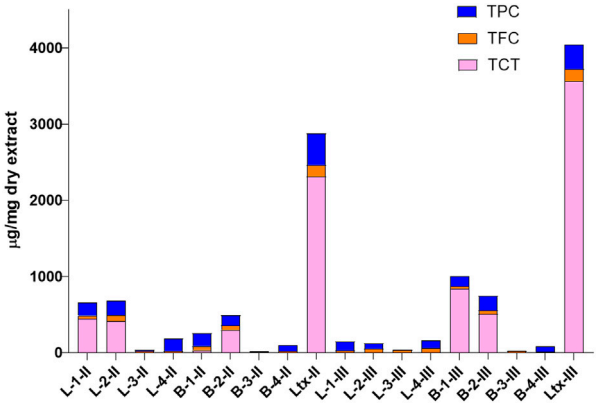


FIGURE 1
Phytochemical characterisation of extracts. TPC: total phenolic compounds, TFC: total flavonoid content, TCT: total condensed tannin content. L (leaves), B (bark), Ltx (latex), II (spring), III (summer). 1 (methanol), 2 (ethanol), 3 (hexane), 4 (water). For individual numerical values and statistical results refer to Supplementary Table S2a.

GC-MS characterisation of essential oils from *C. urucurana*

Essential oils are complex mixtures of volatile secondary metabolites. The unique combination and relative concentrations of these compounds could act as a chemical fingerprint for a particular plant species.

The results of the GC-MS analysis of essential oils from leaves (Eo-L) and bark (Eo-B) are presented in Supplementary Table S1. Essential oils are sesquiterpene-dominant across seasons and tissues (69.7–61.62%). However, marked variability in key sesquiterpenes (e.g., δ-gurjunene, trans-caryophyllene, germacrene D and β-(E)-farnesene) and oxygenated derivatives (e.g., dehydrosesquicineole, sesquicineole and α-bisabolol) reflects distinct chemical profiles among samples. Leaf oils show higher relative abundances of discriminant sesquiterpenes, whereas bark is enriched in oxygenated sesquiterpenes (54.78%). This tissue-dependent chemotype aligns with those of other authors [23], who reported substantially higher oxygenated sesquiterpenes in bark (44.7%) than in leaves (17.7%). These inter-study variations in the monoterpene/sesquiterpene balance and oxygenated fractions support chemical differentiation driven by tissue, seasonality and sampling conditions [24]. While these patterns align with chemotaxonomic differentiation within *Croton* and may help identify potential chemical markers, establishing their diagnostic significance requires broader sampling. Furthermore, the reported anti-inflammatory, antimicrobial and antioxidant activities of several oxygenated sesquiterpenes warrant future investigation, although the present study does not experimentally attribute these effects to individual constituents.

Several compounds are present in both leaf and bark oils, indicating shared biosynthetic pathways. For instance, trans-Caryophyllene, α-trans-Bergamotene, α-Humulene, and β-Bisabolene are found in both Eo-L and Eo-B samples, although their relative concentrations vary. Sesquiterpenes, predominantly β-caryophyllene, germacrene D, β-farnesene, and α-humulene, constitute the major chemical fraction across leaf and bark essential oils [23]. Ecologically, these volatile sesquiterpenic structures play a critical role in plant defense mechanisms, acting as volatile phytoalexins and deterrents against herbivory and phytopathogenic attack [22, 23]. Pharmacologically, these constituents show noteworthy anti-inflammatory, antioxidant, and membrane-stabilising properties that may indirectly attenuate local tissue damage and oxidative stress triggered by

venom enzymes such as phospholipases A_2 and metalloproteinases [24].

Significant differences in compound concentrations were found between samples: β-E-Farnesene and δ-gurjunene were the main components in Eo-L (Summer III), while trans-caryophyllene and germacene-D were the dominant compounds in Eo-L (Spring II). Dehydrosesquicineole was the main compound in Eo-B (Summer III), in agreement with Cândido et al. [22].

Some compounds show tissue or season-specific presence. The case of δ-gurjunene, γ-amorphene, and α-murolene, present at relatively high concentrations in Eo-L (Summer III) were absent in Eo-B (Summer III). Similar results were reported by Turiel et al. [24].

In brief, GC-MS results showed distinct chemical profiles for leaves and bark essential oils, with a significant seasonal variation observed in leaf oil composition.

Phytochemical characterisation of extracts

Figure 1 shows the TPC content for the different extracts evaluated. The overall trend showed that TPC was higher in summer (III) than in spring (II) for all sections evaluated and solvents. Latex in both seasons (II and III) showed the highest TPC content (418.37 ± 17.94 and 325.51 ± 10.77 μg GAE/mg extract), followed by ethanolic extracts of leaves (H-2-II) and barks (C-2-III).

TPC and TFC presented, in general, higher values in summer samples than in spring (Figure 1; Supplementary Table S2a). Latex showed the highest values for both summer and spring seasons, followed by L-2-III, L-4-II and bark extracts (B-1-III and B-2-III). A strong positive linear correlation was observed between TPC and TFC across all evaluated extracts ($r^2 = 0.87$) (Supplementary Table S2b). This tight co-regulation could be attributable to their shared origin in the phenylpropanoid biosynthetic pathway. Key enzymes like phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) are co-ordinately upregulated by seasonal environmental factors (such as solar radiation, temperature, and water stress), resulting in the synchronised accumulation of both general phenolics and specific flavonoids across plant tissues and seasons [12, 46]. Total condensed tannins (TCT) represented the predominant polyphenolic fraction in latex and bark extracts, displaying concentration levels markedly higher than total phenolics (TPC) and total flavonoids (TFC) (Supplementary Table S2a). Specifically, latex collected in summer (Ltx-III) exhibited a TCT value of 3564.2 μg CE/mg extract, which was approximately 11-fold higher than its TPC (325.5 ± 10.8 μg GAE/mg extract) and 23-fold higher than its TFC (149.9 ± 9.5 μg CE/mg extract). Similarly, summer bark methanolic extract (B-1-III) yielded a TCT value of 839.3 ± 41.7 μg CE/mg extract compared to 136.7 ± 8.9 μg GAE/mg extract for TPC and 54.3 ± 1.3 μg CE/mg extract for TFC. Although the vanillin-HCl assay can cross-react with monomeric flavan-3-ols and potentially overestimate tannin concentrations, the predominant presence of polymeric proanthocyanidins in the latex was unambiguously verified by acid-butanolysis and HPLC-DAD analysis.

Several compounds isolated from the *Croton* genus are well-documented, including diterpenes such as clerodanes, proanthocyanidins, alkaloids, phenylbutanoids, flavonoids, and phenols [13, 46], as well as n-alkanes and primary alcohols [47]. In the methanolic extract of bark, compounds such as stigmasterol, β-sitosterol, campesterol, acetyl aleuritic acid, β-sitosterol-O-glucoside, sonderianin, catechin, and gallocatechin have been identified [14, 48, 49].

Leaves of *Croton* species contain flavonol aglycones, flavonol aglycone methyl ethers, flavonol mono- or di-O-glycosides, and tri-O-glycosides [12]. Among the flavonoids present in the latex, proanthocyanidins of varying molecular sizes are its major constituents [50, 51].

As previously shown, latex samples exhibited the highest values for all the evaluated phytometabolites. Condensed tannins have been previously reported as major constituents of latex [51]. Moreover, some authors attribute many of latex´s bioactive properties to these condensed tannins (proanthocyanidins), which have been reported to constitute up to 90% of the dry latex weight in related species such as *C. lechleri* [50–53]. This substantial accumulation of condensed tannins (proanthocyanidins) may hold direct pharmacological implications for the neutralisation of snake venoms. Because of their high molecular weight and dense hydroxyl moiety distribution, condensed tannins bind strongly to catalytic proteins via hydrophobic interactions and hydrogen bonding [21, 45]. This non-specific protein-precipitating ability inactivates major toxic enzymatic components of snake venoms. Additionally, the astringent nature of proanthocyanidins promotes tissue stabilization and localized haemostasis at the envenomation site [20, 25].

Studies on the latex of *C. urucurana* are scarce, but it is reasonable to suppose the presence of similar secondary metabolites found in other *Croton* species. Specifically, the presence of the oligomeric proanthocyanidin SP-303 and a fuco-arabinogalactan as the principal polysaccharide in its exudate have been documented [48]. Additionally, a cytotoxic cyclic peptide, an orbitide, has been identified [27].

Proanthocyanidin analysis

As previously mentioned, proanthocyanidins are major constituents of *C. urucurana* latex; the structural features of oligomeric/polymeric proanthocyanidins have been previously investigated [50, 51]. The study of these compounds usually requires the combination of several techniques to elucidate their structure [54, 55]. One of the procedures developed involves the analysis of butanolysis products by HPLC-DAD [38].

In our work, the butanolysis of Ltx-III (selected based on its alexiteric activity as further explained) yielded an intense red/purple colour (Figure 2A), which suggests the presence of a flavylium moiety within the proanthocyanidin polymer, accounting for the observed red colour. The visible spectrum analysis revealed a maximum absorbance peak at 545 nm (Figure 2B). This is consistent with the visible spectroscopic data reported for the closely related red-coloured anthocyanins, which contain the flavylium/quinone moiety [38, 55]. The HPLC-DAD analysis of Ltx-III did not show remarkable peaks at 254 nm and 280 nm, while no signals were detected at 520 nm (Figure 2C). By contrast, the butanolysis product neither had significant signals at 254 and 280 nm but showed two defined peaks at 520 nm with retention times of 27.1 and 29.9 min (peaks 1 and 2, respectively). The UV-Vis

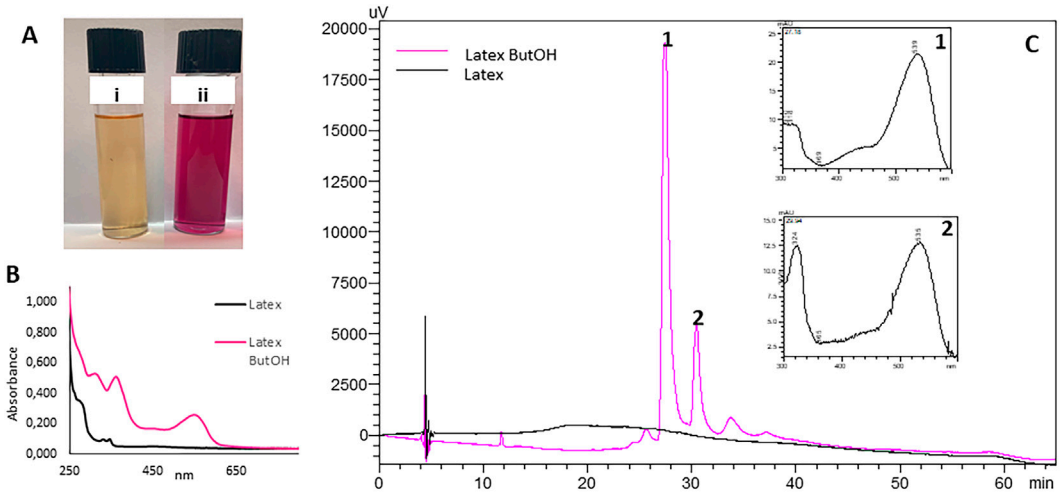


FIGURE 2
(A) Visual appearance of Ltx sample before (i) and after butanolysis (ii). **(B)** UV-Vis spectra for Ltx sample before and after butanolysis. **(C)** HPLC-DAD chromatogram at 520 nm and UV-Vis spectra of Ltx before (black line) and after butanolysis (pink line).

spectral maxima observed at 535 and 539 nm following acid-butanol cleavage are characteristically aligned with cyanidin (Figure 2) [37, 38]. By contrast, other common anthocyanidins generated via depolymerisation of proanthocyanidins, such as delphinidin or pelargonidin, exhibit distinct spectral signatures, with delphinidin displaying a bathochromically shifted maximum (typically $\lambda_{max} \approx$ 543–546 nm) owing to its trihydroxylated B-ring, and pelargonidin displaying a hypsochromically shifted maximum ($\lambda_{max} \approx 520$ nm) [38, 54]. The HPLC-DAD method successfully resolved the reaction products, displaying a predominant chromatographic peak consistent mainly to cyanidin without secondary peaks possessing spectral profiles indicative of delphinidin. These results suggest that *C. urucurana* proanthocyanidins consist predominantly of procyanidin units (catechol B-ring structure) rather than prodelphinidins [50, 51].

Proanthocyanidins have emerged as potent natural alexiteric agents against snake envenomation owing to their multi-target inhibitory mechanisms against major enzymatic toxins. Beyond their recognized antioxidant and anti-inflammatory roles via cyclooxygenase and lipoxygenase inhibition [52, 56, 57], condensed tannins directly neutralise key venom components through non-specific protein precipitation, hydrophobic bonding, and electrostatic cross-linking [21, 45]. Specifically, proanthocyanidins inhibit $sPLA_2$, thereby mitigating indirect haemolysis, while attenuating metalloproteinase activity by stable chelation Zn^{2+} ion. Furthermore, their inhibitory action on venom hyaluronidases restricts matrix degradation, effectively impairing the systemic diffusion of toxins from the local bite site [58].

Alexiteric potential

Screening of alexiteric potential and anti-proteolytic activity by SDS-PAGE

Supplementary Table S3; Supplementary Figures S1, S2 present the results of the screening tests for alexiteric potential (modification of SDS-PAGE pattern) and anti-proteolytic activity (modification of casein pattern) of various extracts, latex, and essential oils from *C. urucurana* collected during spring (II) and summer (III).

Summer samples B-1-III, B-2-III and Ltx-III strongly modified the electrophoretic pattern of *B. diporus* venom; this was evidenced by the loss of characteristic bands (≈15 kDa corresponding to PLA_2, ≈20 kDa corresponding to metalloproteinases and ≈40 kDa corresponding to serine proteases) [3, 6, 41], hence suggesting a strong alexiteric potential. Samples with moderate alexiteric potential included L-1-II, L-2-II, L-4-II, Ltx-II, L-1-III, L-2-III, and Eos samples produced partial attenuation of specific bands (Supplementary Figure S1; Supplementary Table S4).

Six samples (Ltx-III, Ltx-II, B-1-III, B-2-III, Eo-B-III, and Eo-L-III) exhibited the highest anti-proteolytic efficiency, achieving complete preservation (densitometric analysis) of intact casein bands (100% inhibition relative to control) in the presence of *B. diporus* venom (Supplementary Table S3; Supplementary Figure S2).

Nevertheless, minor discrepancies were observed between qualitative SDS-PAGE screening observations and semi-quantitative densitometric measurements (Supplementary Table S3); for instance, samples B-1-II and B-2-II were categorized as inactive in preliminary screening despite maintaining substantial casein band pattern (82.6% and 80.2%, respectively). These divergent results indicate that qualitative visual screening and semi-quantitative densitometry evaluate distinct facets of substrate degradation and venom neutralisation, highlighting that initial screening alone may over- or underestimate the alexiteric potential of plant preparations. Consequently, multi-assay validation *in vitro* assays remain essential to comprehensively elucidate and confirm the inhibitory profile of the most promising extracts.

Evaluating changes in the electrophoretic pattern of venom proteins induced by plant preparations has proven effective as an initial screen for alexiteric activity against snake venoms [40, 43]. Nevertheless, as previously highlighted, potential discrepancies between qualitative gel profiling and other preliminary analytical

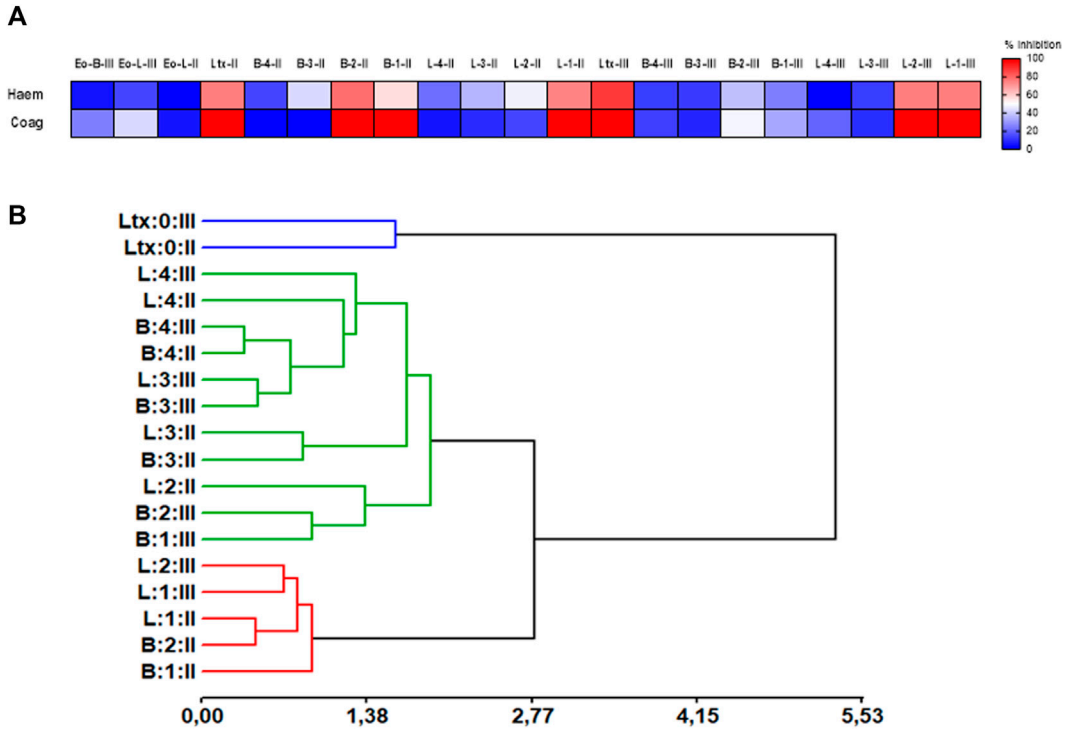


FIGURE 3
(A) Heatmap representation for the inhibition of haemolytic and coagulant activities of samples evaluated, for numerical values refer to Supplementary Table S5. **(B)** Hierarchical cluster dendrogram illustrating the grouping of *C. urucurana* extracts, essential oils, and latex based on phytochemical parameters (TPC, TFC, TCT) and biological activities (%InhC, %InhH). Data were standardised, and the analysis was performed using Euclidean distance and average linkage clustering.

techniques need the implementation of integrated, multi-assay strategies.

Inhibition of coagulant and haemolytic activities of B. diporus venom

The neutralisation efficiency of *C. urucurana* extracts, essential oils and latex against *B. diporus* venom-induced coagulation and indirect haemolysis is visualised as a heatmap in Figure 3A, and individual percentage inhibition values (mean ± standard deviation) are presented in Supplementary Table S4.

Latex samples demonstrated moderate (Ltx-II) to high (Ltx-III) inhibitory potential against the coagulant activity of the venom during both seasons. Notably, Ltx-III exhibited the highest inhibitory potential based on dilutional studies (working at a 1: 32 dilution, Ltx-III exhibited 82.4% inhibition, whereas Ltx-II at a 1: 4 dilution showed 68% inhibition). Bark samples (B-1-II and B-2-II) collected in spring had moderate inhibition over haemolytic activity and strong inhibitory potential against coagulant activity.

Polar solvents (methanol and ethanol) applied to leaves effectively extracted compounds with inhibitory properties in both seasons. Conversely, hexane consistently yielded extracts with the lowest alexiteric (Supplementary Table S3) and inhibitory potentials (Figure 3A; Supplementary Table S5). This low activity is primarily explained by the absence of polar polyphenolic bioactive compounds, particularly proanthocyanidins, total phenolics, and flavonoids, which were non-detectable or present at negligible levels in hexanic fractions (Supplementary Table S2a). The neutralisation of venom enzymes (such as PLA_2, SVMPs, and serine proteases) relies predominantly on hydrogen bonding, electrostatic interactions, and active-site metal chelation, all of which require the hydrophilic hydroxyl moieties characteristic of polar polyphenol [40, 43, 59]. Although lipophilic extractions selectively isolate cuticular wax constituents (e.g., n-alkanes and lipophilic triterpenes) that lack intrinsic anti-enzymatic activity, current evidence suggests this outcome stems from a failure to recover the active polyphenolic complex rather than direct enzymatic interference by non-polar matrix components.

Regarding essential oils, leaf essential oil collected during summer (Eo-L-III) exhibited moderate neutralising efficacy against both coagulant (42.5 ± 1.0%) and indirect haemolytic (14.3 ± 0.3%) activities (Supplementary Table S5).

Our results indicate the presence of discernible variations in the alexiteric potential, primarily influenced by the plant part and the solvent employed for extraction. However, for the most active preparations, particularly latex (Ltx-II and Ltx-III) and polar bark extracts (B-1-II, B-1-III, B-2-II and B-2-III), seasonal variation proved to be a less decisive factor in dictating overall neutralisation capacity.

This functional consistency (inhibition of activities) across phenological stages likely reflects both the constitutive expression of key phenylpropanoid and proanthocyanidin biosynthetic pathways and the intrinsic chemical stability of condensed tannins [10, 43, 59]. In *C. urucurana*, condensed tannins

represent a permanent secondary metabolite reservoir localized within specialized laticifers and cortical tissues, serving as a baseline chemical defense mechanism rather than a transient, stress-induced response [14, 47–49].

Consequently, although climatic stress (elevated temperatures and hydric deficit during summer) could significantly enhance TPC and TCT (Supplementary Table S2a), the observed effect on the inhibition of coagulant and indirect haemolytic activities was not in direct association with seasonal variation. This could suggest that the concentrations of bioactive compounds remained, in summer and spring, above the functional threshold necessary to effectively inhibit both activities.

Cluster analysis

The dendrogram from a hierarchical cluster analysis presented in Figure 3B visually groups extracts based on the similarity of their alexiteric potential and phytochemical characteristics (Eos were not included). For most extract types (aqueous, hexanic, methanolic, and ethanolic), the samples from spring (II) and summer (III) for the same plant part and solvent tend to cluster closely together. This implies that the seasonal variation has a relatively smaller impact on the alexiteric potential of extracts obtained with a specific solvent from a specific plant part. Seasonal changes undoubtedly affect the overall biochemistry of the plant [10]. The extraction process with a specific solvent tends to yield a profile of compounds related to alexiteric potential that is relatively consistent between the two seasons studied.

While the very short horizontal line connecting the spring and summer latex samples suggests a high degree of similarity in their alexiteric potential and phytochemical composition across the two seasons, this observation should be interpreted with caution given that the cluster analysis is based on a limited subset of variables and sample replicates. Moreover, given that these results indicate high functional consistency between the evaluated spring and summer collections, further multi-seasonal studies incorporating controlled environmental parameters (e.g., precipitation, temperature, and soil conditions across multiple years) should be performed to fully determine the impact of seasonal fluctuations on latex bioactive composition. Correspondingly, as latex samples were the ones with best anti-alexiteric properties, Ltx-III was selected for *in vivo* tests.

Inhibition of *Bothrops diporus*-induced dermonecrosis by *C. urucurana* latex

The results above demonstrated the inhibitory activities of *C. urucurana* latex. Its effect as an *in vivo* alexiteric agent was evaluated by topical application of a 3% latex cream. A necrotic lesion (approximately 7 ± 1 mm in diameter after 72 h) was induced via intradermal injection of 150 μg of venom. Histopathological analysis revealed structural differences among experimental groups. In the control group, the epidermis displayed normal morphology, without hyperplasia or alterations in the stratum corneum (Figures 4A–C). The dermis exhibited organised connective tissue, with no inflammatory infiltrate or vascular changes. Skin appendages, including hair follicles and sebaceous glands, remained intact without signs of degeneration. Gomori staining confirmed a homogeneous distribution of collagen and elastic fibres (Figures 4A–C, right section). Similarly, the group treated with *C. urucurana* cream showed slight epidermal thickening with detachment of the stratum corneum, suggesting a proliferative response. The dermis exhibited mild inflammatory infiltrate composed of neutrophils and macrophages. An increase in collagen fibre density was observed, indicating potential fibroblast stimulation. Mild vascular congestion was present, without evidence of thrombosis or haemorrhage (Figures 4D–F). In contrast, the group inoculated with *B. diporus* venom alone exhibited severe alterations. The epidermis showed hyperplasia with cellular disorganisation and detachment of the stratum corneum. The dermis displayed intense inflammatory infiltrate with microabscess formation. Severe vascular damage was evident, including erythrocyte extravasation and focal thrombosis, and the extracellular matrix showed extensive degradation of collagen and elastic fibres, interstitial oedema, and coagulative necrosis (Figures 4G–I). Finally, in the group treated with both venom and *C. urucurana* cream, the epidermis exhibited reduced hyperplasia and disorganisation compared to the venom-only group, suggesting a protective effect of the latex. The dermis showed a moderate inflammatory response, with fewer neutrophils and a higher proportion of macrophages and lymphocytes, indicating a transition toward tissue repair. The extracellular matrix showed better preservation of collagen and elastic fibres, with less degradation, and vascular congestion was less pronounced, and no focal thrombosis was observed (Figures 4J–L).

Semiquantitative histological analysis revealed marked differences among the experimental groups. The control group and the cream-only group showed the lowest lesion scores (0.40 ± 0.55 and 1.40 ± 0.55, respectively). In contrast, *B. diporus* venom inoculation induced severe tissue damage with the highest score (8.80 ± 0.45). Topical application of the *C. urucurana* latex cream reduced lesion severity in the venom-plus-cream group (5.00 ± 1.23), demonstrating an attenuation of histopathological damage.

These results suggest that topical application of *C. urucurana* latex may modulate inflammation and mitigate tissue damage induced by *B. diporus* venom, supporting extracellular matrix preservation and tissue repair. These effects may partly reflect the inhibition of venom enzymatic activities observed in this study. The phytochemical constituents identified may also contribute to tissue protection through antioxidant mechanisms, whereas a possible stimulation of collagen synthesis remains to be investigated. No mortality was observed during the experimental period, consistent with previous findings for *C. lechleri* latex [60]. However, mild histological changes were detected in the cream-only group, and systemic toxicity markers were not assessed; therefore, further studies are required to establish the formulation's safety.

Our findings are in agreement with those of Pieters et al. [58], who reported that crude extracts exhibited significant efficacy in promoting wound contraction, collagen synthesis, and epithelial regeneration, effects attributed to proanthocyanidins. Similarly, another study assessed the wound-healing activity of an ointment containing *C. urucurana* extract in mice, attributing the effect to proanthocyanidins, quercetin, and alkaloids, and demonstrating enhanced healing through increased collagen production, inflammatory cell infiltration, and cytokine expression [18]. Although these studies suggest that other plant preparations may also promote wound healing, our findings apply specifically to the

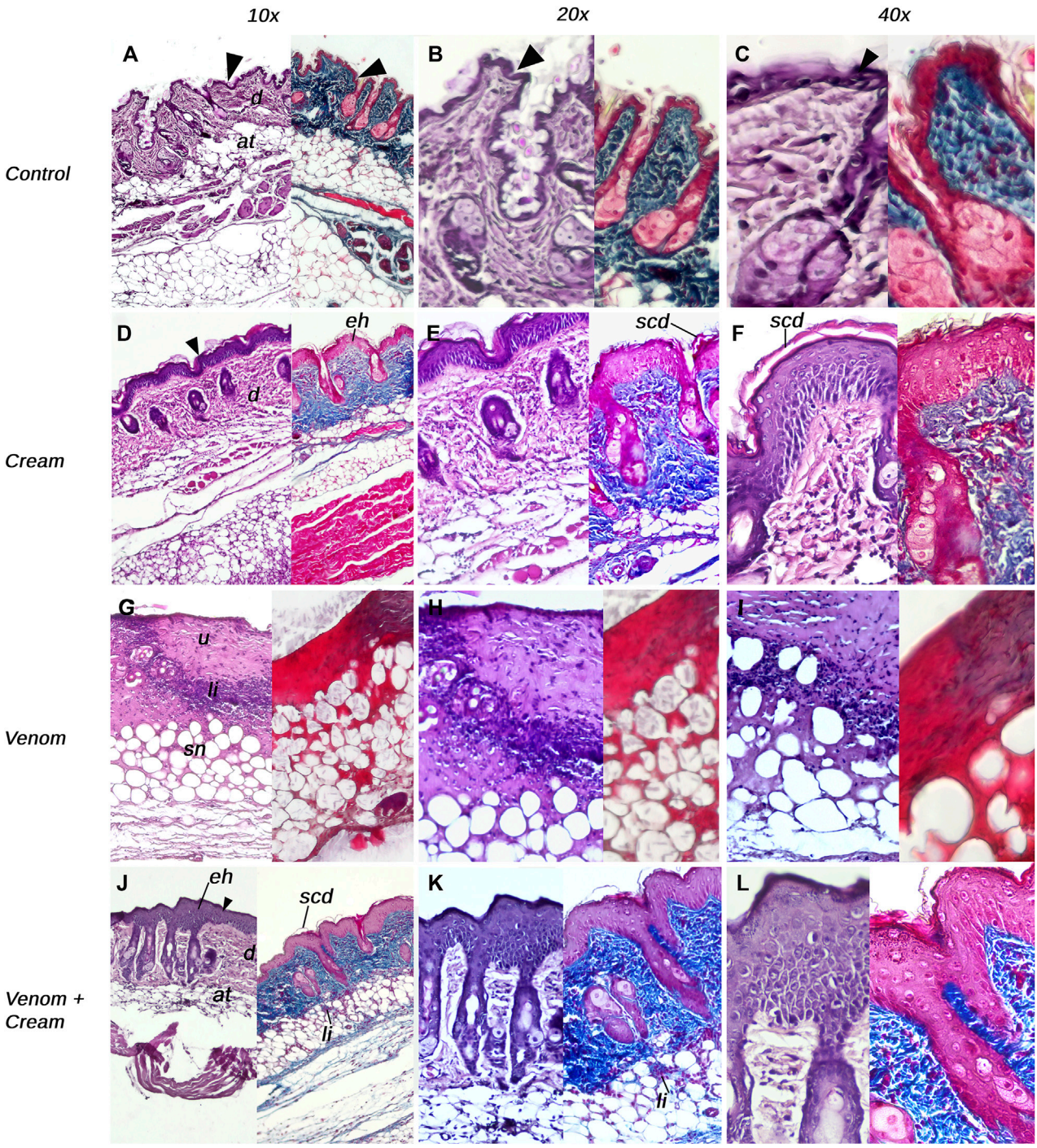


FIGURE 4
Croton urucurana latex-based cream inhibits the dermonecrotic activity of *B. diporus* venom. **(A–C)** Control mouse skin with preserved histoarchitecture. **(D–F)** Mouse skin treated with *C. urucurana* cream, showing epithelial hyperplasia, stratum corneum detachment, and preserved dermis and hypodermis. **(G–I)** Mouse skin injected with *B. diporus* venom, displaying epidermal loss, disorganised dermal fibres, collagen depletion (absence of blue staining), lymphocytic infiltration, and sebaceous necrosis in the hypodermis. **(J–L)** Mouse skin injected with venom and treated with *C. urucurana* cream, showing epidermal hyperplasia, stratum corneum detachment, organised dermal fibers, and minimal lymphocytic infiltration. References: *at* (adipose tissue), *d* (dermis), *scd* (stratum corneum detachment), *eh* (epidermal hyperplasia), *li* (lymphocytic infiltration), *sn* (sebaceous necrosis), *u* (ulcer). Staining: Left images—Hematoxylin-Eosin; Right images—Gomori's trichrome.

latex-based cream; whether extracts from other plant parts provide comparable protection against venom-induced tissue damage remains to be investigated. Collectively, the findings of this study indicate that latex-based formulations of *C. urucurana* exhibit potential as phytotherapeutic agents for alleviating local tissue damage caused by snakebites, hence validating its traditional use in the folk medicine of South American native peoples.

Conclusion

This study highlights the promising alexiteric effects of *C. urucurana* against *B. diporus* venom, providing experimental support consistent with its ethnopharmacological use in South American traditional medicine. Both *in vitro* and *in vivo* assays demonstrated that latex and polar extracts (methanolic and ethanolic) effectively neutralised key enzymatic and toxic actions responsible for local and systemic pathogenesis, including coagulant, indirect haemolytic, proteolytic, and dermonecrotic activities. Topical application of a 3% latex cream, particularly from summer collections (Ltx-III), exhibited inhibitory effects on *in vivo* venom-induced dermonecrosis by suppressing inflammation, preserving extracellular matrix integrity, and accelerating tissue repair.

Preliminary comparison between spring and summer latex samples demonstrated consistent alexiteric efficacy, supporting the stability of latex bioactivity across the studied vegetative stages. Extraction solvent selectivity proved to be the principal determinant of bioactivity; non-polar hexanic fractions consistently lacked efficacy due to the absence of active polyphenolic constituents, whereas polar preparations rich in condensed tannins displayed potent inhibitory potential.

Nevertheless, certain limitations of the present work must be acknowledged. A significant portion of the enzymatic evaluations were conducted under *in vitro* conditions with standardized extract ratios, meaning that detailed dose-response dynamics and precise molecular interaction mechanisms for individual constituents remain to be fully resolved. Furthermore, while these findings support the ethnobotanical application of *C. urucurana*, they do not demonstrate clinical efficacy in human patients.

To build upon these findings, future research should focus on: (i) the bioassay-guided isolation, purification, and structural characterisation of individual bioactive compounds; (ii) evaluating cross-neutralisation efficacy against venoms from other medically important *Bothrops* species; (iii) pharmaceutical formulation optimisation to enhance the chemical stability and topical bio-accessibility of bioactive compounds; and (iv) performing comprehensive pharmacokinetic and toxicological studies to evaluate the potential of standardized *C. urucurana* formulations as supportive adjunctive phytotherapies alongside conventional antivenom administration.

Author contributions

GO: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. JT and FR: Methodology, Investigation, Formal analysis, Data curation. JR: Conceptualization, Writing – original draft, Methodology, Data curation. ED: Writing – review and editing, Formal analysis, Conceptualization. MA: Writing – review and editing, Supervision, Resources, Project administration. AT: Writing – review and editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. All authors contributed to the article and approved the submitted version.

Data availability

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Comité Institucional para el Cuidado y Uso de Animales de Laboratorio. Facultad de Medicina UNNE. The study was conducted in accordance with the local legislation and institutional requirements.

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Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.ebm-journal.org/articles/10.3389/ebm.2026.11343/full#supplementary-material

References

1. WHO. *Snakebite Envenoming* (2023). Available online at: https://www.who.int/news-room/fact-sheets/detail/snakebite-envenoming (Accessed January 20, 2026).

2. de Oliveira VC, Lanari LC, Hajos SE, de Roodt AR. Toxicity of Bothrops neuwiedi complex ("yarará chica") venom from different regions of Argentina (serpentes, viperidae). *Toxicon* (2011) 57:680–5. doi: 10.1016/j.toxicon.2011.01.012

3. Gay C, Sanz L, Calvete JJ, Pla D. Snake venomics and antivenomics of bothrops diporus, a medically important pitviper in northeastern Argentina. *Toxins (Basel)* (2025) 8. doi: 10.3390/toxins8010009

4. Echeverría S, Leiguez E, Guijas C, do Nascimento NG, Acosta O, Teixeira C, et al. Evaluation of pro-inflammatory events induced by Bothrops alternatus snake venom. *Chem Biol Interact* (2018) 281:24–31. doi: 10.1016/j.cbi.2017.12.022

5. Rodríguez JP, Leiguez E, Guijas C, Lomonte B, Gutiérrez JM, Teixeira C, et al. A lipidomic perspective of the action of group iia secreted phospholipase a2 on human monocytes: lipid droplet biogenesis and activation of cytosolic phospholipase a2α. *Biomolecules* (2020) 10:1–20. doi: 10.3390/biom10060891

6. Bustillo S, Fernández J, Chaves-Araya S, Angulo Y, Leiva LC, Lomonte B. Isolation of two basic phospholipases A2 from Bothrops diporus snake venom: comparative characterization and synergism between Asp49 and Lys49 variants. *Toxicon* (2019) 168:113–21. doi: 10.1016/j.toxicon.2019.07.004

7. Lopez GL, Nielsen SA, Nielsen VG, Fusco LS. Procoagulant effects of Bothrops diporus venom: kinetic modeling and role of serine protease activity. *Int J Mol Sci* (2025) 26:9496. doi: 10.3390/ijms26199496

8. Van de Velde AC, Fusco LS, Echeverría SM, Sasovsky DJ, Leiva LC, Gutiérrez JM, et al. Traces of bothrops snake venoms in necrotic muscle preclude myotube formation *in vitro*. *Toxicon* (2022) 211:36–43. doi: 10.1016/j.toxicon.2022.03.008

9. Hamza M, Knudsen C, Gnanathasan CA, Monteiro W, Lewin MR, Laustsen AH, et al. Clinical management of snakebite envenoming: future perspectives. *Toxicon* (2025) 11: 100079. doi: 10.1016/j.toxcx.2021.100079

10. Dellacassa E, Torres AM, Ricciardi GAL, Camargo FJ, Tressens SG, Ricciardi AIA. "Anti-venom activity of medicinal plants from South America," in Utilisation and Management of Medicinal Plants. Gupta VK, Kaul A, (editors). (New Delhi: Daya Publishing House), (2014) 2:1–62.

11. Giovannini P, Howes M-JR. Medicinal plants used to treat snakebite in central America: review and assessment of scientific evidence. *J Ethnopharmacol* (2017) 199: 240–56. doi: 10.1016/j.jep.2017.02.011

12. Oliani J, Ferreira MJP, Salatino A, Salatino MLF. Leaf flavonoids from Croton urucurana and C. floribundus (euphorbiaceae). *Biochem Syst Ecol* (2025) 94:104217. doi: 10.1016/j.bse.2020.104217

13. Salatino A, Salatino MLF, Negri G. Traditional uses, chemistry and pharmacology of Croton species (Euphorbiaceae). *J Braz Chem Soc* (2007) 18(1):11–33. doi: 10.1590/S0103-50532007000100002

14. Lopes MT, Peres P, Monache FD, Pizzolatti MG, Santos ARS, Beirith A, et al. Analgesic compounds of Croton urucurana baillon. In: *Pharmaco-Chemical Criteria Used in Their Isolation* (1998).

15. Wolff Cordeiro K, Aparecida Pinto L, Nazari Formagio AS, Faloni De Andrade S, Leite Kassuya CA, De Cássia Freitas K. Antiulcerogenic effect of Croton urucurana baillon bark. *J Ethnopharmacol* (2012) 143:331–7. doi: 10.1016/j.jep.2012.06.044

16. Cordeiro KW, Felipe JL, Malange KF, do Prado PR, de Oliveira Figueiredo P, Garcez FR, et al. Anti-inflammatory and antinociceptive activities of Croton urucurana baillon bark. *J Ethnopharmacol* (2016) 183:128–35. doi: 10.1016/j.jep.2016.02.051

17. Barbieri DSV, Tonial F, Lopez PVA, Sales Maia BHLN, Santos GD, Ribas MO, et al. Antiadherent activity of schinus terebinthifolius and Croton urucurana extracts on *in vitro* biofilm formation of Candida albicans and Streptococcus mutans. *Arch Oral Biol* (2014) 59:887–96. doi: 10.1016/j.archoralbio.2014.05.006

18. Casao Tdel RL, Pinheiro CG, Sarandy MM, Zanatta AC, Vilegas W, Novaes RD, et al. Croton urucurana baillon stem bark ointment accelerates the closure of cutaneous wounds in knockout IL-10 mice. *J Ethnopharmacol* (2025) 261:113042. doi: 10.1016/j.jep.2020.113042

19. Zago PMJJ, Da SGR, Amaral EC, Barboza LN, Braga FDA, Lorençone BR, et al. Multiple risk factors for heart disease: a challenge to the ethnopharmacological use of Croton urucurana baill. *Evid Based Complement Altern Med* (2021) 2021:1–11. doi: 10.1155/2021/6580458

20. Esmeraldino LE, Souza AM, Sampaio SV. Evaluation of the effect of aqueous extract of Croton urucurana Baillon (euphorbiaceae) on the hemorrhagic activity induced by the venom of *Bothrops jararaca*, using new techniques to quantify hemorrhagic activity in rat skin. *Phytomedicine* (2005) 12:570–6. doi: 10.1016/j.phymed.2004.01.012

21. Kumar A, Banjara RA, Aneshwari RK, Khan J, Bernarde PS. A comprehensive review on recent advances in the use of ethnomedicinal plants and their metabolites in snake bite treatment. *Front Pharmacol* (2025) 16:1548929. doi: 10.3389/fphar.2025.1548929

22. Cândido ACS, Scalon SPQ, Silva CB, Simionatto E, Morel AF, Stüker CZ, et al. Chemical composition and phytotoxicity of essential oils of Croton doctoris S. Moore (euphorbiaceae). *Braz J Biol* (2022) 82:e231957. doi: 10.1590/1519-6984.231957

23. Simionatto E, Bonani VFL, Peres MTLP, Hess SC, Candido ACS, Diraimo DL, et al. Bioactivity and chemical composition of the essential oils of Croton urucurana baillon (euphorbiaceae). *J Essent Oil-Bearing Plants* (2009) 12:250–61. doi: 10.1080/0972060x.2009.10643718

24. Turiel NA, Ribeiro AF, Carvalho EEN, Domingos VD, Lucas FCA, Carreira LMM, et al. Essential oils composition of croton species from the amazon. *Nat Prod Commun* (2013) 8:1471–772. doi: 10.1177/1934578X1300801034

25. Pona A, Cline A, Kolli SS, Taylor SL, Feldman SR. *Review of Future Insights of Dragon's Blood in Dermatology Dermatology Review of Dragon's Blood* (2025). doi: 10.1111/dth.12786

26. Gurgel LA, Sidrim JJC, Martins DT, Filho VC, Rao VS. *In vitro* antifungal activity of dragon's blood from Croton urucurana against dermatophytes. *J Ethnopharmacol* (2005) 97:409–12. doi: 10.1016/j.jep.2004.11.033

27. de Matos Cândido-Bacani P, Ezan F, de Oliveira Figueiredo P, Matos Mde FC, Rodrigues GF, Silva Garcez W, et al. [1–9-NαC]-crourorb A1 isolated from Croton urucurana latex induces G2/M cell cycle arrest and apoptosis in human hepatocarcinoma cells. *Toxicol Lett* (2017) 273:44–54. doi: 10.1016/j.toxlet.2017.03.020

28. Minteguiaga M, Torres AM, Ricciardi Verrastro B, Ricciardi GA, Catalán CAN, Dellacassa E. *In vitro* alexiteric activity of irregular monoterpenes derived from ortho-menthane against Bothrops diporus cope (serpentes: viperidae) venom. *Nat Prod Res* (2026) 40:3269–75. doi: 10.1080/14786419.2025.2463694

29. Warrell DA. Venomous bites, stings, and poisoning. *Infect Dis Clin North Am* (2012) 26:207–23. doi: 10.1016/j.idc.2012.03.006

30. Chiarini F. *Flora Vascular De La República Argentina 10: Dicotyledoneae-Euphorbiaceae*. 1st ed. Buenos Aires, Argentina: Instituto de Botánica Darwinion. (2024). doi: 10.2307/jj.22679657

31. Fernandes FFA, Tomaz MA, El-Kik CZ, Monteiro-Machado M, Strauch MA, Cons BL, et al. Counteraction of bothrops snake venoms by Combretum leprosum root extract and arjunolic acid. *J Ethnopharmacol* (2014) 155:552–62. doi: 10.1016/j.jep.2014.05.056

32. Santoro ML, Do Carmo T, Cunha BHL, Alves AF, Zelanis A, De Toledo Serrano SM, et al. Ontogenetic variation in biological activities of venoms from hybrids between bothrops erythromelas and Bothrops neuwiedi snakes. *PLoS One* (2025) 10:e0145516. doi: 10.1371/journal.pone.0145516

33. Adams RP. *Identification of Essential Oil Components by Gas chromatography/mass Spectrometry*. Carol Stream, IL: Allured Business Media. (2012).

34. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* (1965) 16:144–58. doi: 10.5344/ajev.1965.16.3.144

35. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. *J Taibah Univ Sci* (2015) 9:449–54. doi: 10.1016/j.jtusci.2014.11.001

36. Herald TJ, Gadgil P, Perumal R, Bean SR, Wilson JD. High-throughput micro-plate HCl-vanillin assay for screening tannin content in sorghum grain. *J Sci Food Agric* (2014) 94:2133–6. doi: 10.1002/jsfa.6538

37. Porter LJ, Hrstich LN, Chan BG. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* (1985) 25:223–30. doi: 10.1016/s0031-9422(00)94533-3

38. Pan F, Liu Y, Liu J, Wang E. Stability of blueberry anthocyanin, anthocyanidin and pyranoanthocyanidin pigments and their inhibitory effects and mechanisms in human cervical cancer HeLa cells. *RSC Adv* (2019) 9(19):10842–53. doi: 10.1039/c9ra01772k

39. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (1970) 227:680–5. doi: 10.1038/227680a0

40. Ricciardi VB, Maria Torres A, Ricciardi G, Teibler P, Maruñak S, Barnaba C, et al. The effects of Cissampelos pareira extract on envenomation induced by bothropsdiporus snake venom. *J Ethnopharmacol* (2018) 212:36–42. doi: 10.1016/j.jep.2017.09.015

41. Gay CC, Leiva LC, Maruñak S, Teibler P, Acosta de Pérez O. Acosta de Pérez O. Proteolytic, edematogenic and myotoxic activities of a hemorrhagic metalloproteinase isolated from Bothrops alternatus venom. *Toxicon* (2005) 46:546–54. doi: 10.1016/j.toxicon.2005.06.019

42. Schneider CA, Rasband WS, Eliceiri KW. *NIH Image to Imagej: 25 Years of Image Analysis HHS Public Access* (2012).

43. Torres AM, Camargo FJ, Ricciardi GAL, Ricciardi AIA, Dellacassa E. Neutralizing effects of Nectandra angustifolia extracts against Bothrops neuwiedi snake venom. *Nat Prod Commun* (2011) 6(9):1393–96. doi: 10.1177/1934578x1100600942

44. Theakston RDG, Reid HA. *Development of Simple Standard Assay Procedures for the Characterization of Snake Venoms* (1983). Available online at: https://pmc.ncbi.nlm.nih.gov/articles/PMC2536230/ (Accessed February 10, 2026).

45. Amui SF, Puga RD, Soares AM, Giuliatti S. Plant-antivenom: database of anti-venom medicinal plants. *Electron J Biotechnol* (2011) 14:1–7.

46. Lopes AJJ, Dias MI, Barreira JCM, Barros L, Resende O, Ribeiro Aguiar AC, et al. Phenolic profile of Croton urucurana baill. Leaves, stems and bark: pairwise influence of

drying temperature and extraction solvent. *Molecules* (2025) 25. doi: 10.3390/molecules25092032

47. Pimentel BS, Negri G, Cordeiro I, Motta LB, Salatino A. Taxonomic significance of the distribution of constituents of leaf cuticular waxes of croton species (euphorbiaceae). *Biochem Syst Ecol* (2020) 92:104106. doi: 10.1016/j.bse.2020.104106

48. Milo B, Risco E, Vila R, Iglesias J, Cañigueral S. Characterization of a fucoarabinogalactan, the main polysaccharide from the gum exudate of Croton urucurana. *J Nat Prod* (2002) 65:1143–6. doi: 10.1021/np010188f

49. Gupta D, Bleakley B, Gupta RK. Dragon's blood: Botany, chemistry and therapeutic uses. *J Ethnopharmacol* (2008) 115:361–80. doi: 10.1016/j.jep.2007.10.018

50. Ubillas R, Jolad SD, Bruening RC, Kernan MR, King SR, Sesin DF, et al. SP-303, an antiviral oligomeric proanthocyanidin from the latex of Croton lechleri (Sangre de Drago). *Phytomedicine* (1994) 1:77–106. doi: 10.1016/S0944-7113(11)80026-7

51. Cai Y, Evans FJ, Roberts MF, Phillipson JD, Zenk MH, Gleba YY. Polyphenolic compounds from Croton lechleri. *Phytochemistry* (1991) 30:2033–40. doi: 10.1016/0031-9422(91)85063-6

52. Rao VS, Gurgel LA, Lima-Júnior RCP, Martins DTO, Cechinel-Filho V, Santos FA. Dragon's blood from Croton urucurana (Baill.) attenuates visceral nociception in mice. *J Ethnopharmacol* (2007) 113:357–60. doi: 10.1016/j.jep.2007.06.009

53. Chen Y, Phillipson JDZ-PC. Studies on the anti-tumour, anti-bacterial, and wound-healing properties of dragon's Blood1. *Planta Med* (1994) 60:541–5. doi: 10.1055/s-2006-959567

54. Schofield P, Mbugua DM, Pell AN. Analysis of condensed tannins: a review. *Anim Feed Sci Technol* (2001) 91:21–40. doi: 10.1016/s0377-8401(01)00228-0

55. Ku CS, Mun SP. Characterization of proanthocyanidin in hot water extract isolated from Pinus radiata bark. *Wood Sci Technol* (2007) 41:235–47. doi: 10.1007/s00226-006-0103-8

56. Rauf A, Imran M, Abu-Izneid T, Ul-Haq I, Patel S, Pan X, et al. Proanthocyanidins: a comprehensive review. *Biomed Pharmacother* (2025) 116:108999. doi: 10.1016/j.biopha.2019.108999

57. Nie F, Liu L, Cui J, Zhao Y, Zhang D, Zhou D, et al. Oligomeric proanthocyanidins: an updated review of their natural sources, synthesis, and potentials. *Antioxidants* (2025) 12:1004. doi: 10.3390/antiox12051004

58. Pieters L, De Bruyne T, Van Poel B, Vingerhoets R, Totté J, Vanden Berghe D, et al. *In vivo* wound healing activity of Dragon's blood (croton spp.), a traditional South American drug, and its constituents. *Phytomedicine* (1995) 2:17–22. doi: 10.1016/S0944-7113(11)80043-7

59. Torres AM, Ojeda GA, Angelina E, Bustillo S, Peruchena N, Tonidandel L, et al. The anti-snake activity of Nectandra angustifolia flavonoids on phospholipase A2: *in vitro* and *in silico* evaluation. *J Ethnopharmacol* (2023) 302:115889. doi: 10.1016/j.jep.2022.115889

60. Cevallos-Verdesoto D, Jaramillo-Jaramillo C, Cuesta-Rubio O, Garcia-Simón G, Rojas L. Composición química, actividad cicatrizante y toxicidad del látex de Croton lechleri. *Revista Científica Universidad Del Zulia* (2016) 26:95–103. Available online at: https://www.redalyc.org/journal/959/95945988006/ (Accessed January 20, 2026).