Ciencia, Tecnología y Salud, Vol. 9, Núm. 2, 2022
ISSN: 2410-6356 (impreso) / 2409-3459 (electrónico)
https://doi.org/10.36829/63CTS.v9i2.922

Artículo de revisión / Review Article

Neutralization of toxic activities of *Bothrops asper* snake venom by ethnomedicinal plants used in Central America, with emphasis in Guatemala: a review

Neutralización de actividades tóxicas del veneno de la serpiente Bothrops asper *por plantas etnomedicinales utilizadas en Centroamérica, con énfasis en Guatemala: Revisión*

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Recibido: 29 de mayo 2020 / Revisión: 05 de agosto 2020 / Aceptado: 14 de octubre 2022

Abstract

There are few scientific studies that explore the use of medicinal plants for snakebite envenoming in Central America, although plant-based therapies have been traditionally used in the region. This work reviews the studies conducted in Central America to assess the ability of extracts obtained from plants of local ethnomedical use to inhibit toxic activities of the venom of *Bothrops asper*, the snake responsible for approximately half of the snakebite envenomings in these countries. The search prioritized the description of the plants used in Guatemala, since most of the studies described in this work were conducted in that country, although references to other countries are included. Information concerning secondary metabolites and other pharmacological activities of these plant species, relevant to the treatment of snakebites, was also described. The literature search was conducted in the Google Scholar, PubMed and Scopus databases and completed with locally available literature. It was found that extracts of 12 plant species inhibited the hemorrhagic effect of the venom and three neutralized the edema-forming activity, while inhibition of proteolytic and phospholipase A_2 (PLA_2) activities was achieved by three and one plant species, respectively. Only *Brownea rosa-de-monte* was able to effectively counteract the in vitro coagulant effect of the venom. Some plant extracts screened in Guatemala demonstrated procoagulant or anti-thrombin intrinsic effects that might aggravate the coagulopathy induced by the venom. These findings underscore the need of carrying out scientific studies aimed to validate the inhibitory potential of Central American plant extracts and their metabolites against *B. asper* venom.

Keywords: Snakebite envenoming, traditional medicine, antiophidic plants, antidotes, ethnopharmacology

Resumen

Pocos estudios científicos han explorado el uso de plantas medicinales para el tratamiento del envenenamiento ofídico en Centroamérica, a pesar de que las terapias basadas en plantas son de uso tradicional en la región. Este trabajo recopiló información sobre los estudios realizados en Centroamérica para evaluar la capacidad de extractos de plantas de uso etnomédico para inhibir las actividades tóxicas del veneno de *Bothrops asper*, la serpiente responsable de aproximadamente la mitad de los envenenamientos ofídicos en Centroamérica. La búsqueda priorizó la descripción de plantas utilizadas en Guatemala, ya que la mayoría de los estudios aquí descritos fueron realizados en ese país. También se incluyó la descripción de los metabolitos secundarios y otras actividades farmacológicas de las especies evaluadas, que podrían explicar su uso como antiofídicos. La búsqueda de literatura se realizó en las bases de datos de Google Scholar, PubMed, Scopus, y se completó con literatura disponible localmente. Se determinó que 12 extractos de plantas inhibieron el efecto hemorrágico del veneno y tres el efecto edematígeno; la actividad proteolítica fue inhibida por extractos de tres especies y la fosfolipasa A_2 (PLA_2) por una especie. Solamente *Brownea rosa-de-monte* demostró inhibir efectivamente el efecto coagulante del veneno in vitro. Algunos extractos de las plantas tamizadas en Guatemala demostraron efectos procoagulantes o anti-trombina intrínsecos, que podrían agravar las alteraciones inducidas por el veneno en la coagulación. Estos hallazgos enfatizan la necesidad de validar el potencial de extractos de plantas centroamericanas y sus metabolitos secundarios para neutralizar el veneno de *B. asper*.

Palabras clave: Envenenamiento por mordedura de serpiente, medicina tradicional, plantas antiofídicas, antídotos, etnofarmacología

Introduction

Snakebite envenoming affects as many as 2.7 million people every year in the world, with 81,000 to 138,000 fatalities and 400,000 surviving victims suffering permanent physical and psychological disabilities (Gutiérrez et al., 2017; Kasturiratne et al., 2008). Snakebite envenoming constitutes a serious public health problem in Latin America that primarily affects residents of rural communities, who often have limited access to medical care. It has been estimated that around 5,500 snakebite cases annually reach health centers in Central America, however, the actual number of snakebite victims is likely to be higher, since an unknown number of snake-bitten people are not treated at health facilities, remaining unregistered in the epidemiological data (Giovannini & Howes, 2017; Gutiérrez, 2014).

The most serious cases of snakebite envenoming in Central America are inflicted by species of the family Viperidae, being *Bothrops asper* the species causing the highest number of accidents (Gutiérrez, 2021). This species, locally known as *barba amarilla*, *terciopelo* or *equis*, is distributed from the south of Mexico to the north of Colombia, thus inhabiting all Central American countries, with the exception of El Salvador (Campbell & Lamar, 1989; Solórzano, 2004).

The main therapeutic strategy to treat *B. asper* envenomings relies on the neutralization of the venom toxic components by the parenteral administration of antivenoms, which are antibody preparations obtained from the plasma of horses (Gutiérrez et al., 2017; Rojas et al., 1994; Sánchez et al., 2003). It is known that the systemic toxic actions of *B. asper* venom are effectively neutralized by antibody-mediated inhibition of the most relevant venom components; however, antivenom therapy is less effective to prevent venom-induced local effects (Gutiérrez et al., 1998, 2007; Lomonte et al., 2009). This can be explained by the fact that locally-acting toxins from the venom exert deleterious effects immediately after injection, causing significant damage before people reach hospitals and receive antivenom (Lomonte et al., 1994; Rucavado & Lomonte, 1996). The local pathological damage is associated with permanent tissue loss, impaired skeletal muscle regeneration, functional disabilities and, in severe cases, amputation (Gutiérrez et al., 2018; Jorge et al., 1999;).

Most of the snakebites in Central American countries occur in remote locations far from hospitals, where the access to antivenom therapy and medical care is difficult to reach on time to prevent local tissue damage and to treat the systemic effects of envenoming (Coe & Anderson, 2005; Guerra-Centeno, 2016; Gutiérrez, 2021). Therefore, for many of these snakebite victims the possibility of using treatments applied in the field, rapidly after the bite, may represent a therapeutic alternative. In this regard, the administration of plant extracts is a traditional resource to treat envenomings in several regions of the world (Giovannini & Howes, 2017; Saravia et al., 2001), but the efficacy of such interventions should be rigorously assessed.

In a recent review published by Giovannini and Howes (2017), the traditional use of at least 208 plant species to treat snakebite in Central America was reported. The genera most frequently mentioned were *Piper*, *Aristolochia*, *Hamelia*, *Ipomoea*, *Passiflora* and *Peperomia*. It was also highlighted that the available scientific evidence to support the efficacy and safety is scarce and that more studies are needed to further evaluate the scientific bases for their use. Therefore, it is necessary to explore the efficacy of extracts derived from plants to inhibit venom activities, on the basis of sound scientific experimental methodologies.

This work aims to review the published scientific studies conducted in Central America to assess the ability of plant extracts to inhibit toxic and enzymatic activities of the venom of *B. asper* and the identification of secondary metabolites that might be responsible for the inhibition of the main toxins of the venom. A particular emphasis has been placed on the description of the plants used in Guatemala, since most of the studies described in this work were conducted in that country. Other pharmacological activities of these plant species, relevant to the treatment of snakebites, were also reviewed.

To establish a background reference of studies reporting neutralization of toxic activities of *B. asper* venom by plants collected in Central America, a literature review was conducted in the main available web search engines (Google Scholar, Scopus, and PubMed) for works published in Spanish and English, using as keywords "antiophidic plants" or "medicinal plants", "Central America", "name of the country" + "*B. asper*", "scientific name" + "*B. asper* venom", "toxic activities" "antidotes", "snakebite envenomation" or "snakebite treatment", "proteolytic activity", "phospholipase A_2 activity", "hemorrhage", "coagulant activity" or "anticoagulant activity", "secondary metabolites", "neutralization" or "inhibition". Regarding

the plants traditionally used in Guatemala for treating snakebites, the search detected only two relevant references, particularly the PhD thesis by Hitziger (2016) and the review by Giovannini & Howes (2017). With this information, a data base was built and completed with data collected during the ethnobotanical study conducted in Guatemala by Saravia et al., (2001) and Hay (2002), and other unpublished locally available literature from previous research; the local names were included according to *Flora of Guatemala* (Standley & Steyermark, 1946-1976); and the botanical names updated in Tropicos.org and www.theplantlist.org.

Content

Envenomings caused by the snake *Bothrops asper* in Central America

Envenomings caused by *B. asper* are characterized by a prominent and complex series of local pathological alterations, which appear rapidly after the bite at the site where the venom is injected. Such effects include edema, pain, hemorrhage, myonecrosis, blistering and dermal necrosis (Gutiérrez, Rucavado et al., 2009). Moderate and severe envenomings are associated with systemic alterations, such as systemic bleeding, coagulopathy, hypovolemia, hemodynamic instability, and acute kidney injury; highly severe cases may result in fatality (Gutiérrez, Escalante et al., 2009). Haemostatic alterations, leading to thrombocytopenia, defibrin(ogen)ation and disseminated intravascular coagulation, are frequently observed (Rucavado et al., 2005).

The major components of the venom responsible for inducing this complex clinical picture are snake venom metalloproteinases (SVMPs), phospholipases A_2 (PLA_2) and serine proteinases (SVSMs) (Angulo & Lomonte, 2009; Gutiérrez, 2002). Local effects of envenoming are caused to a great extent by the combined actions of SVMPs and myotoxic PLA_2s (Gutiérrez, Rucavado et al., 2009).

One of the most serious effects induced by the proteolytic activity of SVMPs is hemorrhage, but these enzymes also promote other pathophysiological alterations, such as myonecrosis, blistering, edema, coagulopathy, defibrin(ogen)ation, effects on platelets as well as a proinflammatory activity (Gutiérrez et al., 2010). The action of myotoxic PLA_2S induces extensive damage to the integrity of the skeletal muscle cell membrane, resulting in irreversible cell injury, and it also affects lymphatic vessels and contributes to local inflammation and pain. In addition, the prominent vascular alterations leading to hemorrhage and edema may contribute to ischemia, causing further tissue necrosis (Gutiérrez, Rucavado et al., 2009). PLA_2S also induce additional effects such as inhibition of platelet aggregation, anticoagulation, and cytotoxicity (Angulo & Lomonte, 2009; Díaz et al., 1991; Kini, 2003; Six & Dennis, 2000).

Systemic hemorrhage is primarily caused by the action of P-III SVMPs and is potentiated by the defibrin(ogen)ation induced by the action of SVSPs and C-type lectin-like protein of the venom. Lethality induced by *B. asper* envenoming is the consequence of several combined effects among which the action of P-III SVMPs is especially relevant (Chacón et al., 2015; Gutiérrez, Escalante et al., 2009). Therefore, inhibition of these enzymes by antibodies, natural products or synthetic drugs would considerably diminish the local tissue damage, counteract the systemic effects and prevent development of permanent sequelae in snake bitten people (Carvalho et al., 2013; Félix-Silva et al., 2017; Lomonte et al., 2009; Santhosh et al., 2013).

In 2019 the World Health Organization (WHO) launched a strategy aimed to reduce by half the number of deaths and disabilities caused by snakebite envenomings by the year 2030. Even though the animal-derived antivenoms are the mainstay therapeutic for treating the envenoming, the initiative is promoting the search of complementary therapies that could be administered in the field rapidly after the snakebite, such as natural and synthetic toxin inhibitors (Gutiérrez et al., 2021). In this context, it is relevant to evaluate plant extracts traditionally used in the treatment of snakebite envenomings in different geographical settings, using validated preclinical tests.

Plants with inhibitory activities against *B. asper* venom toxic effects and their active compounds

In a recent review published by Giovannini and Howes (2017), the traditional use of at least 208 plant species to treat snakebite in Central America was reported. Therefore, the subject will not be covered in detail here, except for the plant species used in Guatemala. The search conducted in the consulted sources detected the traditional use of 56 plant species in this

country. Information concerning the traditional form of use, preparation, and route of administration of the plants is shown in Table 1.

There are few published studies conducted in Central America to scientifically validate the therapeutic value of medicinal plants used to inhibit the toxic effects induced by *B. asper* venom. The neutralizing activities of plant extracts were evaluated, in almost all cases, through preincubation-type assays, i.e., incubating venom and plant extract prior to injection in experimental animals or to in vitro testing. Inhibition of toxic effects of the venom were evaluated according to the corresponding experimental settings, depending on the effect being evaluated. Detailed description of the methodologies used to determine the neutralizing activities of the extracts is beyond the scope of this review but interested readers can consult the WHO Guidelines for Production, Control and Regulation of Snake Antivenom Immunoglobulins (2017) and the primary sources cited in this work.

Table 1

Plants traditionally used to treat snakebites in Guatemala

Scientific name	Common name	Part used[1]	Mode of preparation	Mode of use	References
Acanthaceae					
Aphelandra scabra (Vahl) Sm.	Flor de San Julian	NR[2]		NR	Giovannini & Howes, 2017; Hitziger, 2016
Aphelandra heydeana Donn.- Sm.	Flor de San Julian	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Altroemeriaceae					
Bomarea edulis (Tussac) Herb.	Granadillo de monte	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Amaryllidaceae					
Allium sativum L.	Ajo	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Apiaceae					
Eryngium foetidum L.	Samat, culantro	L	Crushed, poultice	Topic (poultice)	Hay, 2002; Saravia et al., 2001
Apocynaceae					Giovannini & Howes, 2017; Hitziger, 2016
Tonduzia stenophylla (Donn. Sm.) Pittier	Chilindrillo	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Aristolochiaceae					
Aristolochia pilosa Kunth	Guapillo	L, Ro, S	Crushed, infusion	Oral, topic (poultice)	Giovannini & Howes, 2017
Aristolochia anguicida Jacq,	Guaco	NR		NR	Hay, 2002 Mejía, 1927 Saravia et al., 2001
Aristolochia maxima L.	Guaco	WP	Decoction	Topic (apply on snakebite)	Aguilar-Girón, 1966

Table 1 (continuation)

Scientific name	Common name	Part used[1]	Mode of preparation	Mode of use	References
Asclepiadaceae					
Asclepias curassavicva L.	Viborana	La	Fresh latex	Topic (apply on snakebite)	Mejía, 1927; Standley & Williams, 1975
Asparagaceae					
Sansevieria hyacinthoides (L.) Druce	Oreja de burro	L	NR	Oral, topic	Kufer et al., 2005; Giovannini & Howes, 2017
*Sansevieria gui*neensis (L.) Willd.	Curarina	WP, L	Fresh parts applied as poultice, leaves as *horchata* for drinking	Topic (poultice), oral	Instituto Indigenista Nacional, 1978; Nicolas, 1999
Sansevieria trifasciata Prain	Curarina, lengua de suegra, oreja de burro rabo de tigre	L	Decoction for drinking Crushed fresh leaves applied as poultice	Oral, topic (poultice) Chewed (poultice)	Ardón Manchamé, 2008; Giovannini & Howes, 2017; Hay, 2002
Asteraceae					
Neurolaena lobata (L.) Cass.	Tres puntas, mano de lagarto	L	Infusion, decoction Chewed with tobacco leaves	Oral, topic (poultice)	Hay, 2002; Saravia et al., 2001
Caricaceae					
Carica papaya L.	Papaya	Fr	Fresh fruit	Topic (place fruit flesh on snakebite to stop poison spread)	Comerford, 1996; Giovannini & Howes, 2017
Commelinaceae					
Gibasis geniculata (Jacq.) Rohweder		NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Tripogandra grandiflora (Donn. Sm.) Woodson	Hoja de fluxión	NR		NR	
Convolvulaceae					
Ipomoea purpurea (L.) Roth	Quiebra-cajete, quilamul	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Euphorbiaceae					
Acalypha arvensis Poepp. *Acalypha alopecuroides* Jacq.	Hierba del cancer Hierba del cancer	Ro L	Crushed, infusion Infusion	Oral (drink), Topic (patch) Oral (drink)	Saravia et al., 2001; Giovannini & Howes, 2017; Mejía, 1927

Table 1 (continuation)

Scientific name	Common name	Part used[1]	Mode of preparation	Mode of use	References
Fabaceae					
Acacia arborea (L.) Willd.	Plumillo, quebraqacho	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Acacia cornigera (L.) Willd.	Subín	R, S	Chewed, decoction	Oral, Poultice, Bath	Giovannini & Howes, 2017; Hay, 2002
Acacia hindsii Benth.	Ixcanal	B	NR	Oral	Aguilar-Girón, 1966; Saravia et al., 2015
Acacia spadicigera Schltldl. & Cham.	Subin, espino blanco	NR		NR	Mejía, 1927
Diphysa robinioides Benth.	Guachipilín	B, L	NR	NR	Mejía, 1927
Erythrina berteroana Urb.	Pito	B	Infusion	Oral	Giovannini & Howes, 2017; Girón et al., 1991; Hitziger, 2016
Gesneriaceae					
Besleria laxiflora Benth.		NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Heliotropiaceae					
Heliotropium indicum L.	Hierba de alacrán	WP	NR	NR	Mejía, 1927
Malvaceae					
Triumffeta lapula	Mozote de caballo	R	NR	NR	Mejía, 1927
Phaseolus coccineus L.	Tzote, verde de maca	L, Fl	Decoction	Topic (cataplasm)	Barreno-Ortiz, 2012; Giovannini & Howes, 2017.
Menispermaceae					
Cissampelos pareira L.	Alcotán, tamagás, curarina de monte	L, R	Decoction	Oral (drink), Topic (poultice)	Ayala, 1999; Giovannini & Howes, 2017; Mejía, 1927; Sandoval & Hay, 2002;
Moraceae					
Dorstenia contrajerva L.	Contrayerba, oreja de conejo, contrahierba	L, R	Decoction	Topic (Bath)	Giovannini & Howes, 2017; Hitziger, 2016; Mejía, 1927; Saravia et al., 2001

Table 1 (continuation)

Scientific name	Common name	Part used[1]	Mode of preparation	Mode of use	References
Nephrolepidaceae					
Nephrolepis cordifolia (L.) C. Presl	Cola de quetzal	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Orchidaceae					
Oeceoclades maculata (Lindl.) Lindl.	Orquidea monje	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Papaveraceae					
Argemone mexicana L.	Chicalote	NR		NR	Mejía, 1927
Petiveriaceae					
Rivina humilis L.	Coxubcan, chile de ratón	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Piperaceae					
Peperomia macrostachya (Vahl) *A. Dietr.*		NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Peperomia quadrifolia (L.) Kunth	Retoño, colchón de peña	NR		NR	Giovannini & Howes, 2017
Piper amalago L.	Cordoncillo	L, R	Crushed fresh leaves, Boil root with other roots	Oral (drink juice of leaves); Topic (bath with boiled root)	Comerford, 1996; Giovannini & Howes, 2017
Piper curvatipes Trel.	Cordoncillo, hoja de piedra, pie de negro	L	Decoction	Topic (bath)	Giovannini & Howes, 2017; Hay, 2002; Saravia et al., 2001
Piper peltatum L.	Cordoncillo, Santa Maria	L	Decoction	Topic (bath)	Giovannini & Howes, 2017; Hay, 2002; Saravia et al., 2001
Piper sempervirens (Trel.) Lundell	Cordoncillo hembra	R	Boil root with roots and leaves of other species	Oral, topic Bathe wound and drink	Comerford, 1996; Giovannini & Howes, 2017
Piper xanthostachyum C. DC	Cordoncillo	L	Decoction	Bath	Hay, 2002
Piper yucatanense C. DC.	Cordoncillo pequeño	L	Decoction	Bath	Hay, 2002
Plantaginaceae					
Scoparia dulcis L.	Escobeta	NR		NR	Giovannini & Howes, 2017; Hitziger, 2016

Table 1 (continuation)

Scientific name	Common name	Part used[1]	Mode of preparation	Mode of use	References
Polypodiaceae					
Phlebodium pseudoaureum (Cav.) Lellinger *Polypodium percussum* Cav.	Calahuala Calahuala	Rh	Decoction	Oral	Giovannini & Howes, 2017 Girón et al., 1991
Pteridaceae					
Antrophyum cajenense (Desv.) Spreng.		NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Vittaria lineata (L.) Sm.		NR		NR	Giovannini & Howes, 2017; Hitziger, 2016
Rubiaceae					
Hamelia patens Jacq. *Psychotria pleuropoda* Donn. Sm.	Coralillo, chichipín Cafecillo, hierba de chinche	L L	Decoction Decoction	Topic (bath) Topic (bath)	Hay, 2002; Giovannini & Howes, 2017; Saravia et al., 2001
Rutaceae					
Citrus aurantifolia (Chirstm.) Swingle	Limón	Fr, L, Ro	NR	Oral	Giovannini & Howes, 2017
Citrus limon (L.) Osbeck	Limón	Fr	NR	NR	Kufer et al., 2005; Giovannini & Howes, 2017
Sapindaceae					
Matayba oppositifolia (A. Rich.) Britton	Zacuayum	B	Boil bark in water with roots of other species	Topic (bath for poisonous bites and stings)	Comerford, 1996; Giovannini & Howes, 2017.
Selaginellaceae					
Selaginella pallescens (C. Presl) Spring	Doradilla	NR		NR	Giovannini & Howes, 2017
Solanaceae					
Nicotiana tabacum L.	Tabaco	Leaf	Crushed, chewed	Topic (patch)	Giovannini & Howes, 2017; Kufer et al., 2005; Saravia et al., 2001

Note. [1]Plant part: B (bark); Fl (flower); Fr (fruits); L (leaf); La (latex); Rh (rhizome); Ro (root); S (stem); WP (whole plant). [2]NR, not reported for Guatemala in the original sources.

Phytochemical analysis of the plant extracts with inhibitory activities were conducted in some of the studies reviewed in this work, aimed at the identification of secondary metabolites responsible for the inhibition of snake venoms and toxins. Among the secondary metabolites with described antivenom properties in the literature are flavonoids, coumestans, alkaloids, steroids, terpenoids, tannins and other phenolic compounds. A detailed description of this topic has been dealt in several reviews (Carvalho et al., 2013; Félix-Silva, et al., 2017; Mors et al., 2000; Santosh et al., 2013; Soares et al., 2005) and will not be considered further here.

In Costa Rica, Castro et al. (1999) showed that 10 plant species (*Bursera simaruba* (L.), *Clusia torresii* Standl., *Clusia palmana* Standl., *Croton draco* Schltdl. & Cham., *Persea americana* Mill., *Phoebe brenesii* Standl, *Pimenta dioica* (L.) Merr., *Sapindus saponaria* L., *Smilax domingensis* Willd., and *Virola koschnyi* Warb.) effectively inhibited the hemorrhagic effect of *B. asper* venom in a mouse model. The secondary metabolites identified in these extracts were catequines, flavones, anthocyanines and condensed tannins, whose antihemorrhagic effect may be explained by their ability to chelate the zinc required by the SVMPs. Although this publication does not provide a source for the reported use of these plants in snakebite envenoming, the selection of the plants included in that publication was based on ethnobotanical information available in Costa Rica (J. M. Gutiérrez, personal communication, August 20, 2021).

Other studies carried out in Costa Rica also identified antihemorrhagic activity in an extract of *Cissampelos pareira* L. (Badilla et al., 2008). Extracts of *Uncaria tormentosa* (Willd.) DC., *Chaptalia nutans* L. Pol. and *Loasa speciosa* Donn. Sm. significantly reduced venom-induced edema in mice when the extracts were injected intraperitoneally one hour before the subcutaneous injection of venom in the hind paw (Badilla et al., 2006). The study published by Núñez et al. (2005) reported the isolation of the active principle 4-nerolidylcatechol in two plants used in snakebite envenomings, *Piper umbellatum* L. and *P. peltatum* Ruiz & Pav. It was shown that 4-nerolidylcatechol significantly reduced the myotoxic and edema-inducing effects of a myotoxic PLA_2 of *B. asper* venom in mice in preincubation assays, but it was ineffective when administered in situ after toxin injection.

In Panama, the evaluation of ethanolic extract of *Brownea rosa-de-monte* P. J. Bergius demonstrated significant inhibition of the coagulant and hemorrhagic effects of *B. asper* venom (Salazar et al., 2014). This study also reported the isolation and structural characterization of quercetrin, one of the active constituents of the extract with anticoagulant activity.

In Guatemala, ethanolic and aqueous extracts obtained from two plants traditionally used to treat snakebite envenoming in the north of this country (*D. contrajerva* and *Neurolaena lobata* (L.) Cass.) (included in Table 1), and one plant used to treat cutaneous leishmaniasis (*Eupatorium odoratum* L.) in the same region of Guatemala (Hay, 2002; Saravia et al., 2001), were analyzed for their ability to neutralize the lethal, hemorrhagic, PLA_2 and coagulant activities of *B. asper* venom (Table 2). Of these, only hemorrhage was minimally inhibited by extracts of *D. contrajerva* and *N. lobata*.

More recent studies conducted with Guatemalan plant specimens (Saravia-Otten et al., 2015, 2017) evaluated the ability of ethanolic extracts of nine plants (*Acasia hindsii* Benth., *Aristolochia maxima* Jacq., *B. simaruba*, *C. pareira*, *Eryngium foetidum* L., *Hamelia patens* Jacq., *P. dioica*, *P. peltatum* and *Sansevieria hyacinthoides* (L.) Druce) to inhibit the coagulant, PLA_2 and proteolytic activities of the venom (Table 2). Six of these plants are used to treat snakebite in the north of Guatemala (*A. maxima* [*Aristolochia sp.*], *C. pareira*, *E. foetidum*, *H. patens, P. peltatum* and *S. hyacinthoides*) (Aguilar-Girón, 1966; Hay, 2002; Saravia et al., 2001), while the traditional use of *P. dioica* was reported in Costa Rica (Castro et al., 1999). The use of *B. simaruba* for this purpose has been reported in Costa Rica and Nicaragua (Castro et al., 1999; Coe & Anderson, 2005). *A. hindsii* is used to treat snakebite envenomings in different regions of the world (Mors et al., 2000) and as an antidote against poisonous bites and stings in Guatemala and Mexico (Aguilar-Girón, 1966; Morton, 1981). Results of in vitro experiments showed that *E. foetidum* and *P. dioica* extracts effectively inhibited the proteolytic effect of the venom, but none of the plants tested inhibited the PLA_2 or coagulant effects (Saravia-Otten et al., 2015, 2017). Phytochemical analysis of these extracts identified secondary metabolites (flavones, anthocyanines, catequines and tannins) whose anti-proteolytic activities have been previously reported. Interestingly, the bark of *B. simaruba* collected in Guatemala showed significant intrinsic proteolytic and PLA_2 activities (Saravia-Otten et al., 2017), a finding that may complicate its possible use in snakebites (Table 2).

Further evaluation of the ethanolic extract obtained from the leaves of *N. lobata* demonstrated

Table 2

Plants screened in Guatemala for their ability to inhibit toxic effects of Bothrops asper *venom*

Plant species	Plant part analyzed[1]	Inhibitory activities tested[2]	Activity inhibited	Other relevant properties in the treatment of snakebite	Reference
Acacia hindsii Benth.	B	P, PLA_2, C	—[9]	Treatment of scorpion stings, anti-inflammatoy and wound healing effects	Aguilar-Girón, 1966; Morton, 1981; Pardo et al., 2012; Saravia et al., 2015, 2017
Aristolochia maxima	B, L	P, PLA_2, C	—	*Aristolochia* species with anti-inflammatory and immunostimulatory effects	Aguilar-Girón, 1966; Giovannini & Howes, 2017; Saravia et al., 2015, 2017
Bursera simaruba (L.) Sarg.	B	P[3], PLA_2[4], C	—	Anti-inflammatory, antibacterial, analgesic, treatment of wounds, poisonwood dermatitis, skin sores and infections	Abad et al., 1996; Arvigo & Balick, 1993; Camporese et al., 2003; Carretero et al., 2008; Jasso de Rodríguez et al., 2006; Junor et al., 2007; Saravia et al., 2017; Sosa et al., 2002; Yasunaka et al, 2005
Cissampelos pareira L.	R	P, PLA_2, C	—	Anti-inflammatory, analgesic, immunomodulatory, muscle relaxant, hemostatic, antiseptic, snake venom inhibition, ulcerogenic	Giovannini & Howes, 2017; Saravia et al., 2015, 2017; Singh et al., 2010; Verrastrero et al., 2018
Dorstenia contrajerva L.	L	L, H, PLA_2, C[5]	—	Treatment of skin conditions (anti-Leishmanial activity)	Giovannini & Howes, 2017; Peraza-Sánchez et al., 2007; Saravia et al., 2001
Eryngium foetidum L.	L	P, PLA_2, C	P[7] P (EA, Ac)[8]	Anti-inflammatory, antibacterial, analgesic, treatment of scorpion bites	Giovannini & Howes, 2017; Küpeli et al., 2006; Paul et al., 2011; Saravia et al., 2017, 2022; Singh et al., 2014
Eupatorium odoratum L.	L	L, H, PLA_2, C[6]	—	Treatment of skin conditions (cutaneous leishmaniasis), hemostatic activity, wound healing, analgesic, anti-inflammatory, antimicrobial activities	Akah, 1993; Hay, 2002; Owoyele et al., 2008; Pandith et al., 2012, 2013; Saravia et al., 2001; Sirinthipaporn & Jiraungkoorskul, 2017; Umukoro & Ashorobi, 2006. Vijayaraghavan et al., 2016, 2017
Hamelia patens Jacq.	L	P, PLA_2, C	—	Relax contracted muscle, effects on the cardiovascular system, treatment of skin conditions, anti-nociceptive, anti-inflammatory, antimicrobial, wound healing	Balick & Arvigo, 1998; Camporese et al., 2003; Giovannini & Howes, 2017; Jiménez-Suárez et al., 2016; Saravia et al., 2015, 2017

Table 2 (continuation)

Plant species	Plant part analyzed[1]	Inhibitory activities tested[2]	Activity inhibited	Other relevant properties in the treatment of snakebite	Reference
Neurolaena lobata (L.) Cass.	L	L, H, P, PLA_2, IH, C[6]	P[7], PLA_2 (as IH)[7] P (Dm, EA, A)[8]	Antiulcerogenic, antimicrobial, antinociceptive, anti-inflammatory, wound healing, treatment of skin conditions.	Cáceres & Cruz, 2019; Giovannini & Howes, 2017; McKinnon et al., 2014; Saravia et al., 2001; Saravia et al., 2021, 2022
Pimenta dioica (L.) Merr.	L	P, PLA_2, C	P[7]	Anti-inflammatory, analgesic, antibacterial, antioxidant, antiulcerogenic, cytotoxic, wound healing and bruises in skin and mucosa like gingivitis	Al-Rehaily et al., 2002; Lorenzo-Leal et al., 2019; Mukhopadhyay et al., 2012; Nagaraj et al., 2017; Onwasigwe et al., 2017; Saravia et al., 2017; Zhang & Lokeshwar, 2012
Piper peltatum L.	L	P, PLA_2, C	—	Analgesic, antiedema, and anti-hemorrhagic, anti-inflammatory, hypertensive, sympathomimetic and activities, inhibition of hyaluronidase from *Bothrops atrox* and PLA_2	Giovannini & Howes, 2017; Michel et al., 2016; Pilco et al., 2019; Saravia et al., 2015, 2017
Sansevieria hyacinthoides (L.) Druce	L	P, PLA_2, C	—	Dressing of sprained ankle, analgesic, antimicrobial, anti-inflammatory, treatment of skin infections and insect bites.	Maroyi, 2013; 2019; Saravia et al., 2015, 2017

Note. [1]Plant part: L (leaf); S (stem); Ba (bark); Ro (root). [2]Inhibitory activities evaluated: L (lethal); H (hemorrhagic); C (coagulant); M (myotoxic); P (proteolytic); PLA_2 (phospholipase A_2); IH (indirect hemolysis). [3]Not evaluated in inhibition assays because of the presence of intrinsic proteolytic, [4]PLA_2, [5]coagulant, [6]anticoagulant activities in ethanolic extracts. [7]Venom activity effectively (≥ 50%) inhibited by ethanolic / aqueous extracts or [8]organic fraction: He (hexane); Dm (dichloromethane); EA (ethyl acetate); Ac (acetone). [9](–) Effective inhibitory activities not detected.

complete inhibition of PLA_2 enzymatic activity (evaluated as indirect hemolysis) and proteolytic activities of *B. asper* venom. However, the extract also showed intrinsic anticoagulant activity, a property that might aggravate the hemostatic alterations induced by the venom in coagulation (Table 2) (Saravia-Otten et al., 2021). The phytochemical profile showed the presence of flavonoids, coumarins, saponins, tannins, sesquiterpene lactones and essential oils in the extract. SDS-PAGE analysis demonstrated changes in the electrophoretic profile, probably due to the formation of insoluble complexes of venom components with plant metabolites.

Analysis of organic fractions obtained from *E. foetidum*, *N. lobata* and *P. dioca* demonstrated that their ability to neutralize PLA_2 and proteolytic activities of the venom was concentrated in their polar fractions. Phytochemical analysis of the bioactive fractions identified flavonoids, saponins, essential oils, coumarins, alkaloids, tannins and sesquiterpene lactones in their composition (Saravia-Otten et al., 2022). Docking analysis of compounds from these plants revealed high

affinity interactions of previously identified secondary metabolites of these plants with key residues for the catalytic activity of BaP1 (a metalloproteinase) and Mtx-I (a PLA_2), as well as interactions with the hydrophobic channel of Mtx-II (a PLA_2 homologue devoid of enzymatic activity but inducing myotoxicity). These findings provide support for the use of *E. foetidum*, *N. lobata* and *P. dioca* to treat the local effects induced by *B. asper* envenoming, since it has been shown that in vitro inhibition of proteolytic activity (mainly mediated by SVMPs) by some plant extracts correlates with inhibition of the hemorrhagic effect in animal models (Patiño et al., 2013; Preciado et al., 2018), although more studies with in vivo models are necessary to further explore this possibility. It has also been shown that plant extracts with anti-PLA_2 activity partially neutralized myotoxicity and edema induced by the venom of snakes of the genera *Bothrops* and *Crotalus* in animal models (Pereañez et al., 2014; Posadas et al., 2019).

Ethnopharmacology of the plants studied in Guatemala

There is a strong tradition among Guatemalan people in using medicinal plant antidotes, which are prepared with local plants by traditional healers (*chamanes, curanderos, medicos brujos, herbolarios*), according to their ancestral recipes (Hay, 2002; Saravia et al., 2001). The information presented here is focused on the plants that demonstrated inhibitory activities against toxic effects of *B. asper* venom in preclinical tests (*E. foetidum*, *N. lobata*, and *P. dioica*), the traditional mode of use and preparation, and is aimed to provide available information that supports their possible use for snakebite envenomings. Other relevant properties of the plants in the treatment of snakebite are shown in Table 2.

E. foetidum is used to treat snakebites in Guatemala, Nicaragua, and Belize (Balick & Arvigo, 2015; Coe & Anderson, 2005; Giovaninni & Howes, 2017; Hay, 2002). Decoction and infusion of the leaves are administered as a beverage to the snake bitten patient in Nicaragua (Coe & Anderson, 2005), while in Guatemala the crushed leaves are applied on the wound as a poultice (Hay, 2002; Saravia et al., 2001). Traditional use of this species is supported in part by results obtained using in vitro assays and animal models showing anti-inflammatory, analgesic, and antibacterial properties of the leaf extract (Almeida et al., 2001; García et al., 1999; Küpeli et al., 2006; Paul et al., 2011; Sáenz et al., 1997), all relevant for the inhibition of local effects of *B. asper* venom. This species is also used to treat snakebite and scorpion sting envenomings in India (Singh et al., 2014).

N. lobata is traditionally used to treat snakebites in Guatemala, Belize, and Nicaragua (Balick & Arvigo, 2015; Coe & Anderson, 2005; Hay, 2002; Saravia et al., 2001); it is also used to treat skin conditions in Guatemala (Berger et al., 2001; Cáceres et al., 1998). Infusion and decoction of the leaves are drink by snake bitten victims in Nicaragua and Guatemala (Coe & Anderson, 2005; Hay, 2002; Saravia et al., 2001). Decoction is also administered topically to the wound in the north of Guatemala, and the leaves used to prepare the infusion or fresh leaves chewed with tobacco by the patient are used as a poultice to cover the wound (Hay, 2002). Ethnomedical use of stems, branches, and aerial parts of *N. lobata* to treat snake envenoming has also been reported in Colombia (Otero et al., 2000). Extracts and organic fractions obtained from this species have demonstrated to have antiulcerogenic (Gracioso et al., 2000), antimicrobial (Lentz et al., 1998), antinociceptive (Gracioso et al., 1998) and anti-inflammatory properties (Giovannini & Howes, 2017; McKinnon et al., 2014; Walshe-Roussel et al., 2013). These activities are relevant for the treatment of snakebites, since they might counteract some of the local effects induced by *B. asper* venom, promote successful wound healing, and prevent sequelae resultant from bacterial infections that would delay, or even impair, the healing process (Nayak et al., 2014).

P. dioica is used in the treatment of a number of medical conditions in traditional medicine of Mexico, Central and South America, the Caribbean, India, Middle East, Asia and Europe (Rao et al., 2012; Zhang & Lokeshwar, 2012). However, there is a lack of published information concerning the ethnobotanical use of *P. dioica* to treat snakebite envenoming. The work published by Castro et al. (1999) reported that extracts from the leaves collected in Costa Rica completely neutralized the hemorrhagic activity induced by the venom of *B. asper*, which correlates well with the ability shown by the ethanolic extract of Guatemalan specimens to inhibit the proteolytic activity of the venom (Saravia et al., 2017). Ethnomedicinal uses of the plant and pharmacological activities found on its extracts may explain those findings, suggesting the potential usefulness of *P. dioica* in snakebite envenoming in Central America. The crushed fruits of the plant (Allspice) are applied to bruises, sore joints and for myalgia in Guatemala, while the leaves are traditionally used for treatment

of high blood pressure in Central America, as well as treatment for menstrual cramps and abdominal pain in Caribbean communities (Zhang & Lokeshwar, 2012). All parts are used in folk medicine, but the most studied parts have been its leaves and berries. The eugenol present in the essential oil form berries and leaves has been reported to have anti-inflammatory activities (Zhang & Lokeshwar, 2012). Hypotensive action of aqueous extract of leaves was demonstrated during in vivo studies (Suárez et al., 1997). Studies carried out in Saudi Arabia with the aqueous suspension showed significant antiedema and antiulcerogenic activities in vivo (Al-Rehaily et al., 2002). Essential oil of the berries collected in Mexico demonstrated antimicrobial and potential anti-inflammatory activities in vitro (Lorenzo-Leal et al., 2019). Antibacterial activity was also detected in leaf and bark extracts in India (Asha et al., 2013) as well as antioxidant activity in Jamaica (Rao et al., 2012). Other medicinal properties attributed to this species in India are as an aid in the healing of wounds, bruises (Mukhopadhyay et al., 2012), and gingivitis (Nagaraj et al., 2017).

Decoction of stembark of *A. hindsii* is used to treat poisonous bites and scorpion stings in México and Guatemala (Aguilar-Girón, 1966; Morton, 1981), while decoction of the whole plant is topically used for antiinflamatory and wound healing purposes in the south of Guatemala (Pardo Villegas et al., 2011). *A. maxima* is reported to be used as 'antiophidic' in Guatemala; decoction of the whole plant is applied locally on the snakebite (Aguilar-Girón, 1966; Hay, 2002; Mejía, 1927). *C. pareira*, *D. contrajerva* and *H. patens* are used with the same purpose in Guatemala, Nicaragua and Panamá (Coe & Anderson, 2005; Duke, 1970; Gupta et al., 1993; Hay, 2002; Joly et al., 1987; Sandoval & Ayala, 1999; Saravia et al., 2001), *P. peltatum* in Guatemala and Nicaragua and *S. hyacinthoides* in Guatemala and Belice (Balik & Arvigo, 2015; Coe & Anderson, 2005; Hay, 2002; Kufer et al., 2005). Ethnobotany and ethnopharmacology of those species and the scientific evidence that support their traditional use in snakebite envenoming in Central America was extensively reviewed by Giovannini and Howes (2017); other relevant properties of these plants for the treatment of snakebite are shown in Table 2. However, detailed information concerning *B. simaruba* and *E. odoratum* was not included in that review, and therefore will be addressed in this work.

The bark and whole plant of *B. simaruba* is traditionally used in Nicaragua to treat snakebite in the form of a decoction (Coe & Anderson, 2005). Extracts obtained from the bark totally inhibited the hemorrhagic activity of *B. asper* venom (Castro et al., 1999). This species is used in traditional medicine in Central America for deep and superficial wound healing, skin sores and poisonwood dermatitis (Arvigo & Balick, 1993; Balick & Arvigo, 1998). The therapeutic properties described for this species are relevant to the treatment of local effects induced by the venom, since extracts from the leaves and bark showed anti-inflammatory activity in mice (Abad et al., 1996; Carretero et al., 2008; Noguera et al., 2004; Sosa et al., 2002). Antibacterial activity was demonstrated in the essential oil from fruits and stems (Junor et al., 2007), bark (Camporese et al., 2003), leaves, twigs, and stems (Yasunaka et al., 2005). The plant also has analgesic activity (Jasso de Rodríguez et al., 2006).

The leaves of *E. odoratum* are used to treat skin conditions in Guatemala (Hay, 2002) and other regions of the world (Hanphakphoom et al., 2016). The pressed extract of the leaves is traditionally used in Nigeria to arrest bleeding from cuts and as antiseptic for wound dressing (Akah, 1993), while the leaf or stem decoction of the plant can be used in pulmonary hemorrhage (Singha, 1965). Hemostatic activity of extracts of the leaf has been shown in animal models (Akah, 1993; Pandith et al., 2012). This plant has also wound healing activity (Pandith et al., 2013; Sirinthipaporn & Jiraungkoorskul, 2017; Vijayaraghavan et al., 2017), as well as analgesic, anti-inflammatory and antimicrobial properties (Owoyele et al., 2008; Taiwo et al., 2000; Umukoro & Ashorobi, 2006; Hanphakphoom et al., 2016; Vital & Rivera, 2009; Vijayaraghavan et al., 2016). Therefore, it appears that both leaves and stem of *E. odoratum* may mediate different pharmacological actions which could be useful in snakebite envenoming.

Conclusions

There is a compelling need to search for novel therapeutic alternatives for snakebite envenoming that would complement the action of animal-derived antivenoms. Inhibitory compounds present in plants constitute a valuable source of novel venom inhibitors, and the long-standing ethnobotanical traditions in many regions of the world constitute a highly relevant source of information for exploring the inhibitory potential of these natural products. All plants included in the scientific studies reported in this review have

ethnomedical uses or pharmacologic activities relevant to the treatment of snakebites (i.e. anti-inflammatory, antiedema, antibacterial, wound healing, hemostatic or analgesic). These activities may explain their traditional use against the local effects caused by viperid snakebite envenomings, but their capacity to effectively inhibit venom toxins, and therefore, to neutralize their pathophysiological effects, needs to be further evaluated using sound scientific methodologies. Antihemorrhagic activity was demonstrated in 11 plant species in Costa Rica, while *B. rosa-de-monte* showed antihemorrhagic and anticoagulant activities in Panama. Venom-induced edema in mice was inhibited by *U. tormentosa*, *C. mutans* and *L. speciosa* in Costa Rica. In Guatemala, *E. foetidum*, *N. lobata* and *P. dioica* were effective to inhibit the proteolytic and PLA_2 activities of the venom under experimental conditions, suggesting that they may be effective to neutralize their toxic effects. Moreover, docking studies provided evidence for the ability of some secondary metabolites from these plants to bind with high affinity to SVMP and PLA_2. The capacity of some Guatemalan plants, and secondary metabolites present in these plants, to inhibit venom toxic activities in vivo needs to be further evaluated in preclinical assays.

It is important to point out that from all the plant species screened, only *B. rosa-de-monte* was able to effectively counteract the in vitro coagulant effect (potentially defibrin(ogen)ating in vivo) of *B. asper* venom. Moreover, some of the ethanolic extracts of the plants screened in Guatemala demonstrated procoagulant or anti-thrombin intrinsic effects. Since systemic bleeding is one of the main clinical features of envenomings by *B. asper*, the possibility of these plant extracts to complicate this aspect of envenoming should be carefully considered in the in vivo experimental studies. Likewise, the purification and assessment of efficacy of isolated components from these extracts should be explored.

In conclusion, it is important to harness the rich ethnobotanical tradition on the use of plants for snakebite management in Central America, by testing the efficacy of plant extracts to inhibit toxic and enzymatic activities of *B. asper* and other venoms in the region using validated preclinical assays. These studies should also explore the traditional ways by which these extracts are administered and use these protocols in the experimental settings. It is also important to identify the metabolites in the plants responsible for the inhibition of the SVMPs, PLA_2s and SVSPs to better understand the mechanisms of inhibition, and for exploring the potential use of such metabolites or molecules derived from them for the design of more specific inhibitors of the toxins responsible for the main hallmarks of *B asper* envenoming.

Contribution of authors

Coordinación, elaboración y revisión del Documento: todos los autores
Diseño de la recolección de datos o del trabajo en campo: PS-O, AC
Recolección o contribución de datos o realización del trabajo de campo: todos los autores
Limpieza, sistematización, análisis o visualización de datos: todos los autores
Participación en análisis de datos, estructura y en la escritura del documento: todos los autores

Suplementary materials

This article does not have supplementary materials.

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