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Review Article

Neutralizing effects of Brazilian plants against snake venoms

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CONTENTS

Abstract

Medicinal plants constitute a very rich source of natural inhibitors of animal toxins, and may be used: 1) to study the mechanism of action of toxins and inhibitors; 2) to treat ophidian envenomation as a supplementary and/or alternative therapy; and 3) as models to design new drugs of interest in clinical medicine. Several Brazilian plants have been utilized in folk medicine as antiophidians. However, only a few species have been scientifically investigated and still fewer have had their active components isolated and characterized both structurally and functionally. This article presents a review of Brazilian species showing neutralizing properties against snake venoms which have been assayed in the research laboratory and characterized ethnopharmacologically in terms of: 1) the part of the plant used as antidote; 2) the respective genus and family; and 3) the main pharmacological properties related to inhibition of toxic and enzymatic activities of snake venoms and isolated toxins.

Introduction

Snake bite envenomation constitutes a relevant public health hazard in Latin America (1, 2). Most accidents are due to species of the genus *Bothrops*, although envenomations by *Crotalus* spp. also occur throughout the region, particularly in South America, where they are usually associated with severe systemic envenomations (1, 3, 4).

Snake venoms are complex mixtures of proteins, including phospholipase A_2 (PLA_2; EC 3.1.1.4), myotoxins, hemorrhagic metalloproteases and other proteolytic enzymes, cytotoxins, cardiotoxins and others (5, 6). The pathophysiology of snake bite envenomation involves a complex series of events that depend on the combined action of these venom components (7). Local tissue damage (hemorrhage, myonecrosis and edema) is one of the most dramatic effects of envenomation by Crotalinae and Viperinae snakes. Local edema, a typical manifestation of *Bothrops* envenomation, usually in addition to pain, is due to the action of the venom upon mastocytes, kininogens and phospholipids, culminating in the release of endogenous mediators (8, 9).

Snake venoms contain many proteolytic enzymes that degrade a variety of natural substrates, such as casein, fibrinogen, collagen and others. Hemorrhagic toxins are responsible for the degradation of proteins from the extracellular matrix or alterations in blood coagulation (10, 11) and require a divalent metal ion for their activity.

Muscle necrosis is an important local effect induced by several snake venoms, sometimes resulting in an irreversible loss of tissue and occasionally requiring amputation of the affected limb. Myonecrosis may be due to an indirect action as a consequence of vessel degeneration and ischemia caused by hemorrhagic metalloproteases, or a direct effect of myotoxic PLA_2 homologues upon plasma membranes of muscle cells. Recently, there has been an increased interest in PLA_2 homologues from snake venoms as the agents responsible for myonecrosis. Besides playing a digestive role in phospholipid hydrolysis, they may also exert a wide variety of effects, including pre- or postsynaptic neurotoxicity, cardiotoxicity, myotoxicity, platelet aggregation induction or inhibition, induction of edema, hemolysis, anticoagulation, convulsions and hypotension (7, 12-15).

Envenomation by snake bites is frequently treated by parenteral administration of horse- or sheep-derived polyclonal antivenoms aimed at the neutralization of toxins (16). However, despite the widespread success of this therapy, it is important to search for new venom inhibitors,

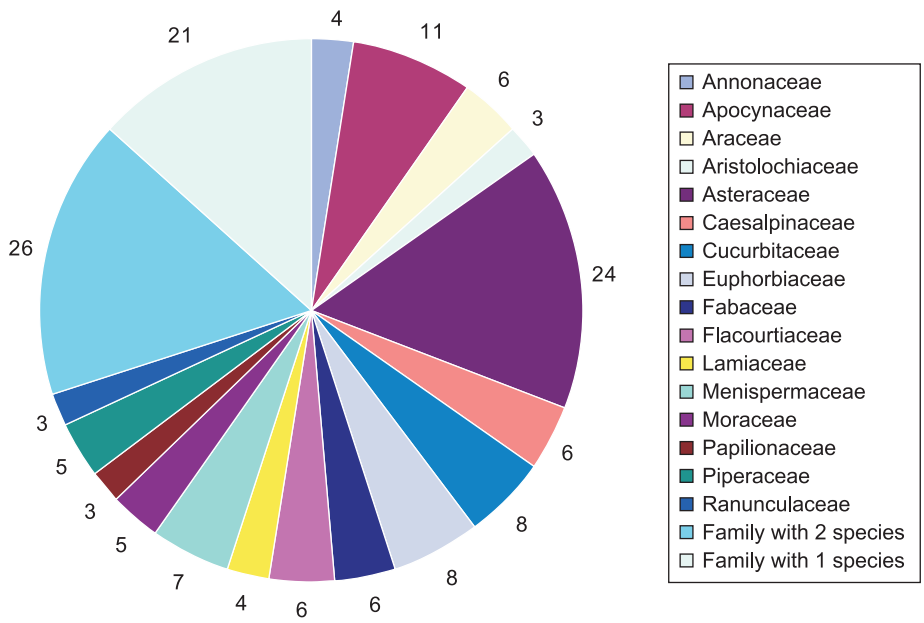

Fig. 1. Families of Brazilian plants believed to be active against snake venoms.

either synthetic or natural, that would complement the action of antivenoms. Natural venom inhibitors may be particularly valuable for neutralizing local tissue damage. Medicinal plant extracts, a rich source of natural inhibitors and pharmacologically active compounds, have been shown to antagonize the activity of some venoms and toxins.

In some parts of the world inhabited by venomous snakes, several plant species are employed in popular medicine for the treatment of ophidian envenomation (17-21). However, some plants believed to display antiophidian activity proved to be inactive in clinical and pharmacological experiments (19). Several species of Brazilian flora are popularly known as antiophidian, but only a few species have been scientifically investigated and still fewer have had their active principles isolated and characterized (21).

This review describes Brazilian plant species displaying antiophidian potential which were scientifically assayed and characterized in ethnopharmacological studies in terms of the part of the plant used as antidote, its respective genus and family, and inhibition of the main pharmacological, toxic and enzymatic activities of snake venoms and isolated toxins.

Brazilian plants active against snake bite envenomation

Many plants are used in popular medicine to treat snake bite envenomation (18, 21), but few attempts have been made to investigate the scientific background of these assertions by means of controlled experiments, the nature and activity of their active chemical principle(s) and possible mechanisms of action.

Ethnopharmacological studies

This section deals with ethnopharmacological studies on Brazilian plant species categorized by family, as part of their validation as inhibitors of the effects of venom toxins. Published results of ethnopharmacological surveys carried out in the last 20 years with plants from Brazil's biodiversity have indicated that certain geni of different families are promising sources of natural new antiophidian drugs.

Rizzini *et al.* (22) listed 83 species of plants belonging to 34 families and Hashimoto (23) listed 66 species of plants belonging to 31 families that are used in Brazilian folk medicine as antidotes against snake venoms. Among these families are the Annonaceae, Apocynaceae, Araceae, Asteraceae, Caesalpinaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Menispermaceae, Moraceae and Piperaceae families, with over 5 species belonging to each family (Fig. 1). It is important to point out that 20 species are cited in both surveys.

Several species traditionally used by the Brazilian population have already been chemically studied, allowing correlations between structure and biological activity of isolated components assayed against the lethal action of *Bothrops jararaca* venom (21, 24, 25). Table I shows that although 152 plant species are utilized in Brazilian folk medicine as snake venom antidotes, only 18 species (12%) have been scientifically validated (Fig. 2A). These data are reflected in popular medicine, where only 3

Table I: Brazilian plants active against snake venoms and ethnopharmacological data.

Plant species	Plant family	Plant source(s)	Antiophidian properties	Snake species	Ref.
Baccharis trimera	Asteraceae	Leaves Stems	Antihemorrhagic Antiproteolytic Antiedema	*B. alternatus* *B. jararacussu* *B. moojeni* *B. neuwiedi*	67
Bredemeyera floribunda	Polygalaceae	Roots	Antilethality	*B. jararaca*	40, 41
Cascaria mariquitensis	Flacourtiaceae	Leaves	Antihemorrhagic Antiproteolytic	*B. neuwiedi*	46
Casearia sylvestris	Flacourtiaceae	Leaves Roots	Anti-PLA_2 Antiedema Antimyotoxic Antihemorrhagic Antilethality	*B. alternatus* *B. jararacussu* *B. moojeni* *B. neuwiedi* *B. pirajai* *C. d. terrificus*	35, 44, 45, 73
Cordia verbenacea	Boraginaceae	Leaves	Antiedema Antimyotoxic Antihemorrhagic	*B. jararacussu*	47
Curcuma longa	Zingiberaceae	Roots	Antihemorrhagic Antilethal	*B. jararaca* *C. d. terrificus*	28
Eclipta prostrata	Asteraceae	Leaves Stems	Anti-PLA_2 Antihemorrhagic Antiproteolytic Antimyotoxic	*C. rhodostoma* *B. jararaca* *B. jararacussu* *L. muta*	33, 34, 63
Harpalyce brasiliana	Fabaceae	Roots	Antimyotoxic Anti-PLA_2 Antiproteolytic	*B. jararacussu*	31, 32, 74
Jatropha elliptica	Euphorbiaceae	Subterranean system Root	Anti-PLA_2 Antihemorrhagic	*B. alternatus* *B. jararacussu* *B. moojeni* *B. neuwiedi* *C. d. terrificus*	This review
Mandevilla velutina	Apocynaceae	Subterranean system Stem Leaves	Antineurotoxic Antimyotoxic Antihemorrhagic Antiedema Anti-PLA_2 Antiproteolytic	*B. alternatus* *B. jararacussu* *B. moojeni* *B. pirajai* *C. d. terrificus*	48, 50
Mandevilla illustris	Apocynaceae	Subterranean system	Antineurotoxic Antilethal Anti-PLA_2	*C. d. terrificus*	49
Marsypianthes chamaedrys	Lamiaceae	Aerial parts	Antilethal Antiedema Anticlotting	*B. jararaca* *B. jararacussu* *B. alternatus* *B. neuwiedi* *B. insularis* *B. atrox* *L. muta* *C. d. terrificus*	25, 36-38
Mikania glomerata	Asteraceae	Roots	Antiedema Anti-PLA_2 Antihemorrhagic	*C. d. terrificus* *B. jararacussu*	This review
Sapindus saponaria	Sapindaceae	Leaves Fruits Corns	Antihemorrhagic Antiedema Antimyotoxic Anti-PLA_2	*B. alternatus* *B. jararacussu* *B. moojeni* *B. pirajai* *C. d. terrificus*	This review
Scleria pterota	Cyperaceae	Leaves	Antihemorrhagic Anticoagulant Antiedema Anti-PLA_2	*B. alternatus* *B. jararacussu* *B. moojeni* *B. neuwiedi* *C. d. terrificus*	This review

Continued

Table I Cont.: Brazilian plants active against snake venoms and ethnopharmacological data.

Plant species	Plant family	Plant source(s)	Antiophidian properties	Snake species	Ref.
Swartia corrugata	Fabaceae	Stems	Antilethality Antihemorrhagic	*B. atrox*	75
Tabernaemontana catharinensis	Apocynaceae	Roots	Antineurotoxic Antimyotoxic Antilethality	*C. d. terrificus* *C. d. collineatus* *C. d. cascavella*	42, 43, 76, 77
Torresea cearensis	Papilionaceae	Stems	Antilethality	*C. d. terrificus*	78

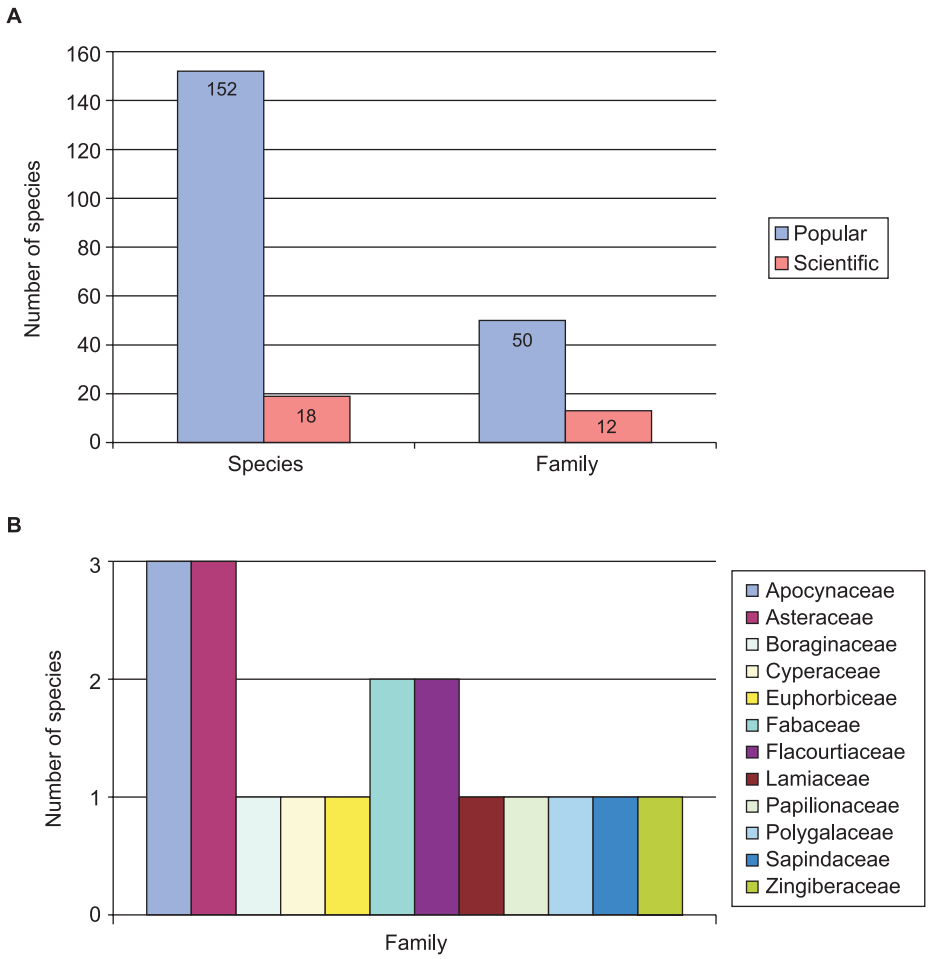

Fig. 2. **A.** Plant species and families popularly considered to be and scientifically validated as antagonists of snake venoms. **B.** Number of Brazilian species by plant family with scientific reports on neutralizing effects of snake venoms.

species have been studied from the Apocynaceae and Asteraceae families and only 2 from the Fabaceae and Flacourtiaceae families (Fig. 2B).

Ethnopharmacological and chemosystematic studies may help us to identify new plant species active against snake bite envenomation. Melo *et al.* (26) evaluated the antiophidian activity of two Brazilian plants know as "arnica". They used extracts in dichloromethane and ethanol (1:1) from *Lychnophora pinaster* leaves, as well as from *Solidago chilensis* aerial parts. Neither species suppressed the edema induced by *Bothrops alternatus* venom, indicating that not all popularly utilized plants are indeed scientifically valuable.

The antiophidian activity of several other plant species in general use in Brazilian communities was also investigated scientifically. Cherdchu *et al.* (27) showed that the aqueous extract of *Curcuma* rhizomes abolished the neuromuscular inhibition produced by a neurotoxin from *Naja naja siamensis* snake venom. When the *Naja* venom and *Curcuma* extract were administered

independently by different routes, no prolongation of survival time was observed, indicating that deactivation occurred *in vitro*. A potent antivenom, named arturmerone, active against the hemorrhagic and lethal activities of *B. jararaca* and *Crotalus durissus terrificus*, respectively, was isolated from *Curcuma longa*, a plant commonly used in popular Brazilian medicine (28).

Nakagawa *et al.* (29) isolated the prenylated pterocarpans cabenegrin A-I and A-II. These compounds are thought to be responsible for the antidotal effect of a locally well-known antivenom remedy ("*Específico Pessoa*") manufactured and sold in north and northeast Brazil. Unfortunately, the botanical identity of the roots is unknown (30). More recently, another pterocarpan, edunol, was isolated from the roots of *Harpalyce brasiliana ("erva-de-cobra").* Both edunol and a synthetic derivative were assayed and shown to inhibit the myotoxic activity of *Bothrops jararacussu* venom, while the synthetic compound showed higher anti-PLA_2 and antiproteolytic activities (31). According to da Silva *et al.* (32), the presence of those pterocarpans in the root of *H. brasiliana* suggests that the phytomedicine "*Específico Pessoa*" was prepared from the plant "*erva-de-cobra*" and not from "*cabeça-de-negro*", as previously reported (29).

Mors *et al.* (33) reported the *in vitro* neutralization of lethal and myotoxic activities of South American rattlesnake venom by extracts and components of *Eclipta prostrata*. This plant is known as "*erva botão*" in Brazil and is also used in China. Several compounds, including wedelolactone and dimethylwedelolactone, were identified as its antiophidian constituents (21). An *E. prostrata* extract and wedelolactone also antagonize hemorrhage induced by crotalid venoms. The potential of butanolic extracts of *E. prostrata* from Thailand to act against *Calloselasma rhodostoma* (Malayan pit viper) venom was demonstrated by their ability to neutralize the lethal and hemorrhagic effects of this venom (34).

Pereira *et al.* (35) described the oral pretreatment of mice against twice the lethal dose of *B. jararaca* venom. Extracts of *Phylanthus klotzschianus*, *Casearia sylvestris* and *Apoleia leiocarpa* conferred 100% protection for up to 48 h after administration. Ruppelt *et al.* (25, 36) and Pereira *et al.* (37) have reported the antiophidian activity of *Marsypianthes chamaedrys* extracts, showing that mice can be protected against the toxicity of *B. jararaca* venom by previous oral administration of the leaf extract. This extract was also effective in protecting against edema and fibrin clotting induced by several Brazilian snake venoms (37, 38).

Bredemeyera floribunda is another remedy for the treatment of snake bite envenomation used in Brazilian folk medicine (39). In laboratory tests, bredemeyerosides showed snake venom antidote activity (24). *B. floribunda* triterpenoid saponins showed high activity against the lethality of *B. jararaca* snake venom (40, 41).

In the rural community of Assis-SP Brazil, the root bark latex of *Tabernaemontana catharinensis* ("*leiteiro*") is topically applied to the site of the snake bite and is believed to neutralize the effect of the venom. Batina *et al.* (42, 43) reported that the aqueous extract and an isolated quaternary alkaloid (MMV) from the ethanolic extract of *T. catharinensis* inhibited the lethal and myotoxic activities of *C.d. terrificus* venom.

Borges *et al.* (44, 45) showed that the aqueous extract of *C. sylvestris* was efficient in neutralizing edema and the myotoxic, proteolytic and hemorrhagic (Fig. 3) activities of *Bothrops* venoms and isolated toxins. Aqueous extracts from the leaves of *Casearia mariquitensis*, a plant found in open pastures in Brazil, were tested for their ability to inhibit hematological and hemostatic effects induced by neuwiedase, a 22-kDa class P-I metalloprotease from the venom of the South American pit viper *Bothrops neuwiedi pauloensis* (46). The results clearly indicate that *C. mariquitensis* extract contains metabolites that are potent inhibitors of metalloproteases. These findings may open up the possibility of isolating and characterizing inhibitory molecules from this plant in the future.

The methanolic extract from *Cordia verbenacea* significantly inhibited paw edema and myotoxicity induced by *B. jararacussu* snake venom and by its major basic PLA_2 homologues, namely bothropstoxins I and II (47).

Aqueous extracts of *Mandevilla velutina* and *Mandevilla illustris* are able to inhibit several classes of toxins (Fig. 3) due to specific interactions with crude venom components, as well as with purified neurotoxins from *C.d. terrificus* venom (48-50). *M. velutina* is a perennial plant endemic to the Brazilian savanna, but condemned to extinction due to interruption of the natural propagation process. Recently, this plant was targeted for conservation as part of the Brazilian biodiversity (51) in light of the fact that about 80% of the Brazilian savanna has been devastated (52). The cloning of plants *in vitro* has been encouraged focusing on the preservation and multiplication of elite genotypes to preserve the pharmacological effects (53-55).

Phospholipase A_2 inhibitors (PLIs)

Phospholipase A_2 (PLA_2) comprises a class of enzymes which catalyze the hydrolysis of membrane glycerophospholipids at the sn-2 position to release fatty acids and lysophospholipids. When the fatty acid is arachidonic acid, complementary metabolism leads to proinflammatory and procoagulating mediators such as prostaglandins, leukotrienes, thromboxanes and PAF. PLA_2 enzymes have been identified and characterized in mammalian tissues, as well as in arthropod and snake venoms. Recently, mammalian PLA_2 enzymes have been implicated as playing a role in numerous pathophysiological processes, including rheumatism and osteoarthritis, asthma, psoriasis, septic shock and adult respiratory distress syndrome (56, 57).

The toxicity of snake venom is often addressed (58) in terms of the activities of PLA_2 enzymes, which are major components thereof. In addition to the digestion of phosphoglycerides, PLA_2 enzymes exhibit a wide range of effects, including neurotoxicity, cardiotoxicity, myotoxicity,

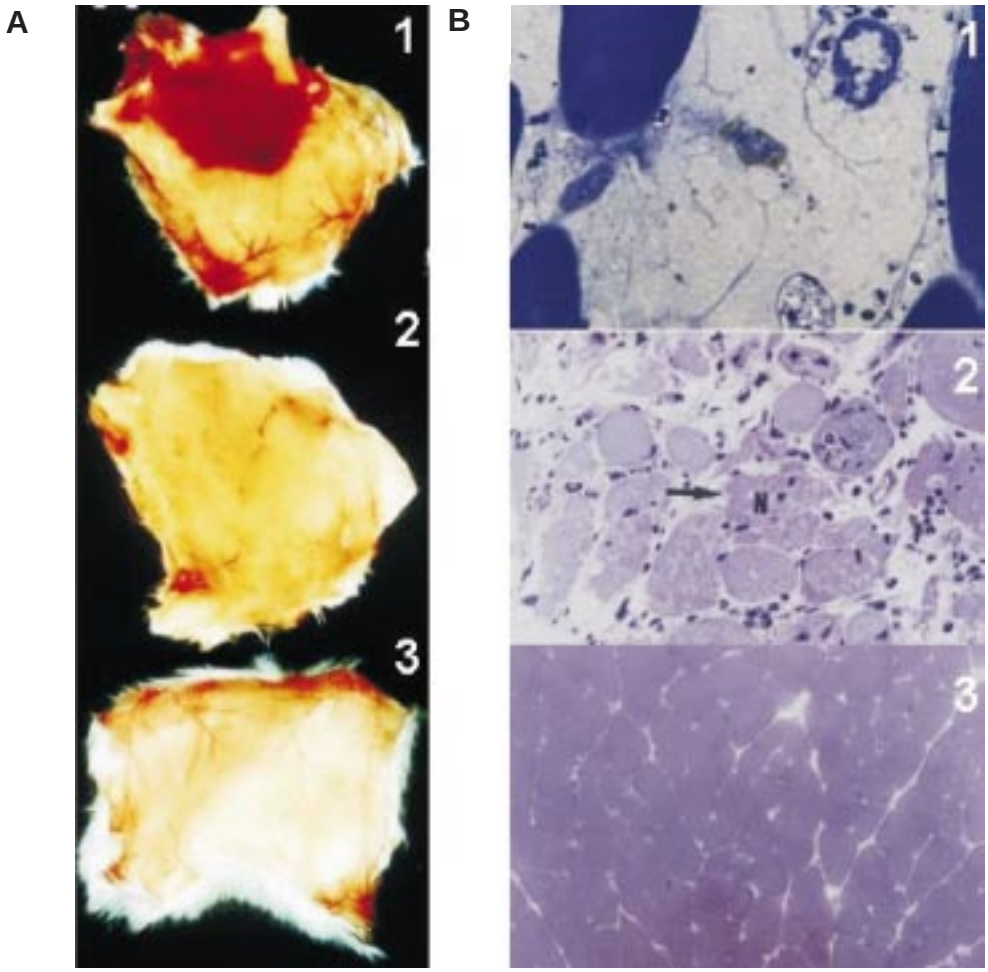

Fig. 3**.** Inhibition of hemorrhagic and myotoxic activities of *Bothrops moojeni* venom by aqueous extracts of *Casearia sylvestris* and *Mandevilla velutina*. **A.** Antihemorrhagic effect. Lanes: 1 - *B. moojeni* venom (30 μg/50 μl); 2 - *C. sylvestris* extract + *B. moojeni* venom (10:1, w/w); 3 - *M. velutina* extract + *B. moojeni* venom (10:1, w/w), after 30-min incubation at 37 ºC. **B.** Antimyotoxic effect. Lanes: 1 - *B. moojeni* venom (30 μg/μl); 2 - *B. moojeni* myotoxic Lys49-PLA_2 (MjTX-II, 50 μg/50 μl); 3 - *C. sylvestris* or *M. velutina* extracts + MjTX-II (10:1, w/w), after 30-min incubation at 37 ºC. N: myonecrosis.

necrosis, anticoagulation, hypotension, hemolysis, hemorrhage and edema (12). Modulating proinflammatory lipid mediator production by inhibiting PLA_2 activity represents a potential strategy for the development of new drugs for the treatment of inflammatory diseases, and a series of PLIs has been isolated from natural sources, such as marine organisms, snakes and plants (20, 59-61).

Vishwanath *et al.* (62) studied the interaction of *Vipera russelli* PLA_2 with the *Aristolochia* component aristolochic acid. This inhibitor appears to recognize subtle differences that occur in the structure of PLA_2 isoforms of snake venoms (20). Wedelolactone and MMV, compounds isolated from *E. prostrata* and *T. catharinensis* plant extracts, respectively, effectively inhibit the myotoxic activity of *C.d. terrificus* (33, 43), *B. jararacussu*, *B. jararaca* and *Lachesis muta* (63) venoms, as well as various isolated myotoxic PLA_2 enzymes (64).

In 2001, Bernard *et al.* (65) carried out an ethnopharmacological study associated with bioinformatics, reporting 68 potential anti-PLA_2 plant extracts. Phytochemical comparative analyses showed that betulin and betulinic acid, present in several species studied, were potent PLIs.

C. sylvestris, which has been proven effective in neutralizing the myotoxic and edematogenic properties of various toxic PLA_2 enzymes from snakes (44), may also serve as a source of PLIs that could be used in the future as potent antivenom compounds. An extract was shown to be active against animal venom PLA_2 enzymes belonging to classes I, II and III, with the greatest inhibition observed against venom enzymes from *Bothrops* and *Crotalus* geni. Biondo *et al.* (48) also showed that the aqueous extract from *M. velutina* had a broad spectrum of inhibitory activity against the toxic, enzymatic and pharmacological activities of snake venoms and isolated

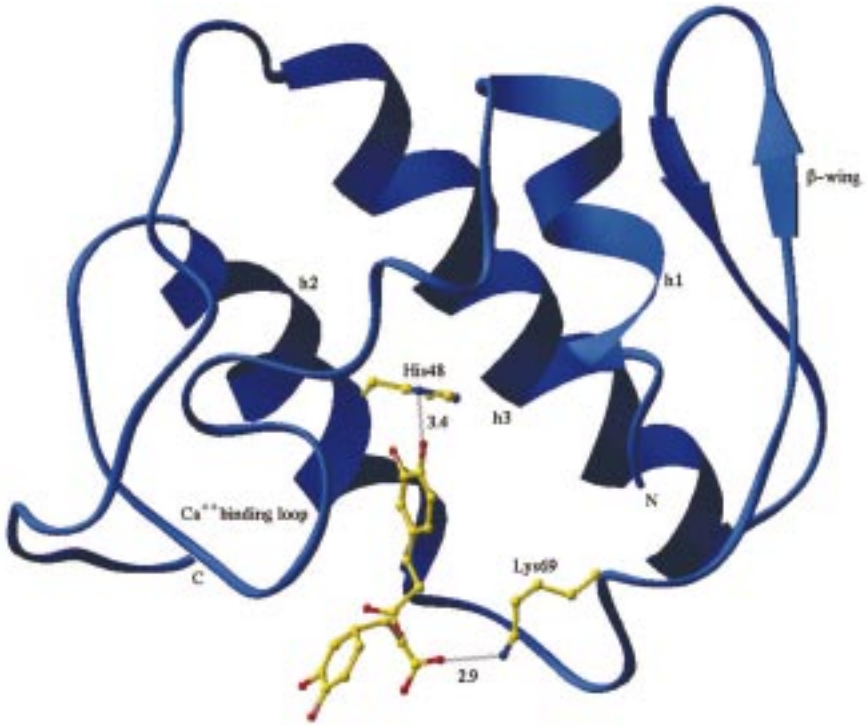

Fig. 4. Molecular simulation of the interaction between PLA_2 isolated from *Bothrops moojeni* (Lys49 MjTX-II) and the PLA_2 inhibitor from *Cordia verbenacea* extract (rosmarinic acid).

toxins. However, this extract was more specific for *Crotalus* venom and its neurotoxic basic PLA_2 when compared with *Bothrops* acidic PLA_2. The inhibition of *N. naja* PLA_2 by the aqueous extract of *M. velutina* was also reported (50).

A *C. verbenacea* methanolic extract and its component rosmarinic acid (Cv-RA) inhibit the edema and myotoxicity induced by *B. jararacussu* crude venom and its main PLA_2 homologues, indicating that this plant has potential antiophidian activity (47). Rosmarinic acid was much more effective in inhibiting edema induced by the Lys49 PLA_2 bothropsotoxin-I (BthTX-I) than that induced by its isoform Asp49 BthTX-II. However, it was similarly effective in neutralizing the myotoxicity of both toxins. This polyphenolic acid was also isolated from several plants belonging to the Boraginaceae and Laminaceae families. Rosmarinic acid was modeled into the hydrophobic channel leading to the active site of *Bothrops moojeni* Lys49-PLA_2. After energy minimization, rosmarinic acid remained in the hydrophobic channel with a hydroxyl group on one of the aromatic rings bound to His48 and the carboxyl group bound to Lys69 (Fig. 4).

Studies on the interactions of plant PLIs with snake venom PLA_2 enzymes provide an additional dimension to understanding the mechanism of action of PLA_2 enzymes. These interactions occur at specific sites and hence are capable of recognizing subtle structures that are responsible for biological activities. These investigations support the hypothesis that a PLA_2 toxin should have a specific site on its structure to induce specific pharmacological and enzymatic effects (20, 66). No information on the structure or inhibitory spectrum is yet available on these active principles. However, it is important to note that the presence of PLIs opens up the possibility to search for plant inhibitors of snake venoms for therapeutic purposes.

Molecular structures of inhibitors

The structures and origin of some plant antiophidians already isolated and characterized are shown in Table II and Figure 5.

Steroid and terpenoid compounds

The activities of the extracts of *E. prostrata* are due to three active components: wedelolactone and the steroids sitosterol and stigmasterol. Among the active constituents, wedelolactone appears to be the most potent (20, 21).

Compound MV-8608, characterized as a new aglycone steroid, and compound MV-8612, a steroidal glycoside, were isolated from *M. velutina* and showed antiinflammatory activity against snake venom PLA_2 enzymes (50).

Table II: Compounds isolated from Brazilian antiophidian plants.

Plant species	Family	Compound(s) isolated	Ref.
Apuleia leiocarpa	Leguminoseae-Caesalpinaceae	β-Amyrin Apulein	24
Baccharis trimera	Asteraceae	Clerodane diterpenoid (Bt-CD)	67
Betula alba	Betulaceae	Betulin and betulinic acid	65
Bredemeyera floribunda	Polygalaceae	Bredemeyerosides B and D	21, 24, 40, 41
"*Cabeça de Negro*"	-	Cabenegrins A-I and A-II	29
Cordia verbenacea	Boraginaceae	Rosmarinic acid	47
Curcuma longa	Zingiberaceae	Ar-turmerone	21, 28
Cynara scolymus	Asteraceae	Cynarin	24, 25
Derris sericea	Papilionaceae	Derricidin	24
Derris urucu	Papilionaceae	2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine	79
Dorstenia brasiliensis	Moraceae	Bergapten	21, 24
Eclipta prostrata	Asteraceae	Wedelolactone, sitosterol and stigmasterol	21, 24, 33, 63, 64, 80
Harpalyce brasiliana	Fabaceae	Edunol	21, 31
Mandevilla velutina	Apocynaceae	Steroids	50, 80
Mikania glomerata	Asteraceae	Coumarin	21, 24
Periandra mediterranea	Papilionaceae	Triterpenes, sterols, sitosterol and periandrins (mixt.)	24
Phyllanthus klotzchianus	Euphorbiaceae	Quercetin	24
Phyllanthus klotzchianus	Euphorbiaceae	Rutin	24
Piper caldense	Piperaceae	Caldensin	81
Silybum marianum	Asteraceae	Silymarin	24
Tabernamontana catharinensis	Apocynaceae	Quaternary alkaloid (MMV)	42, 43
Vernonia condensata	Asteraceae	Caffeic acid and derivatives Chlorogenic acid	24

Clerodane diterpenoid (Bt-CD), an active component isolated from *Baccharis trimera,* was identified as 7α-hydroxy-3,13-clerodadiene-16,15:18,19-diolide, and found to exhibit antiproteolytic and antihemorrhagic properties against snake venoms. Bt-CD exhibited full inhibition of hemorrhage and proteolysis caused by *Bothrops* snake venoms (67). Among the triterpenoids, the pentacyclic triterpenes betulin and betulinic acid, extracted from *Betula alba,* showed anti-PLA_2 activity (65).

A triterpenoid saponin, bredemeyeroside D, was isolated from *B. floribunda* and showed significant protection against lethality due to *B. jararaca* snake venom (40). A new triterpenoid saponin, bredemeyeroside B, has also been isolated from the roots of *B. floribunda*. Bredemeyeroside B showed snake venom antidote activity in laboratory tests (41).

Several pentacyclic triterpenes are associated with antivenom activity, including oleanolic acid, lupeol, ursolic acid, taraxerol, taraxasterol, α,β-amyrin and friedelin. However, tetracyclic triterpenes with activity against snake venom have not been found in plants. It has been suggested that, in the case of antivenom and antiinflammatory activity, a 5-ring triterpene structure with a specific conformation is essential for the active pharmacophore (21).

Phenolic compounds

Wedelolactone, a coumestan isolated from *E. prostrata*, was found to be active against South American crotalid venoms of *C.d. terrificus* (33), *B. jararaca*, *B. jararacussu* and *L. muta* (63), *Crotalus viridis viridis* and *Agkistrodon contortrix* (64). A series of synthetic wedelolactone analogues antagonized the creatine kinase (CK) release induced by *B. jararacussu* venom at 30 μM (68). The active synthetic coumenstan displayed an oxidation pattern on the D-ring similar to that of wedelolactone, which is oxidized at C-8 or C-9, suggesting that this ring oxidation pattern is related to the antiophidian activity (68).

Pterocarpans prenylated in the A-ring are described as very active compounds. As previously mentioned, two pterocarpans, cabenegrins A-I and A-II, are the main ingredients of "*Específico Pessoa*", a folk medicine used in Brazil against snake bite envenomation. Another pterocarpan denoted edunol was isolated from the root of *H. brasiliana*, a plant used against snake bite envenomation. This compound was subsequently obtained by synthesis and showed antimyotoxic, antiproteolytic and PLA_2-inhibitory properties (31).

Other synthetic prenylated and benzylated pterocarpans active against snake venom have also been obtained. The synthetic inhibitors, showing antimyotoxic, antiproteolytic and anti-PLA_2 activities, were designed based on the natural inhibitor edunol (32). The authors demonstrated that the antivenom activity of edunol could be improved by substituting the prenyl group at position 4 for a benzyl group, while activity was completely eliminated by the introduction of an additional group in position 10 (31). The active natural compounds cabenegrins A-I and A-II and edunol have a C-5 side-chain on the A-ring, while the synthetic derivatives without the A-ring side-chain were inactive (31).

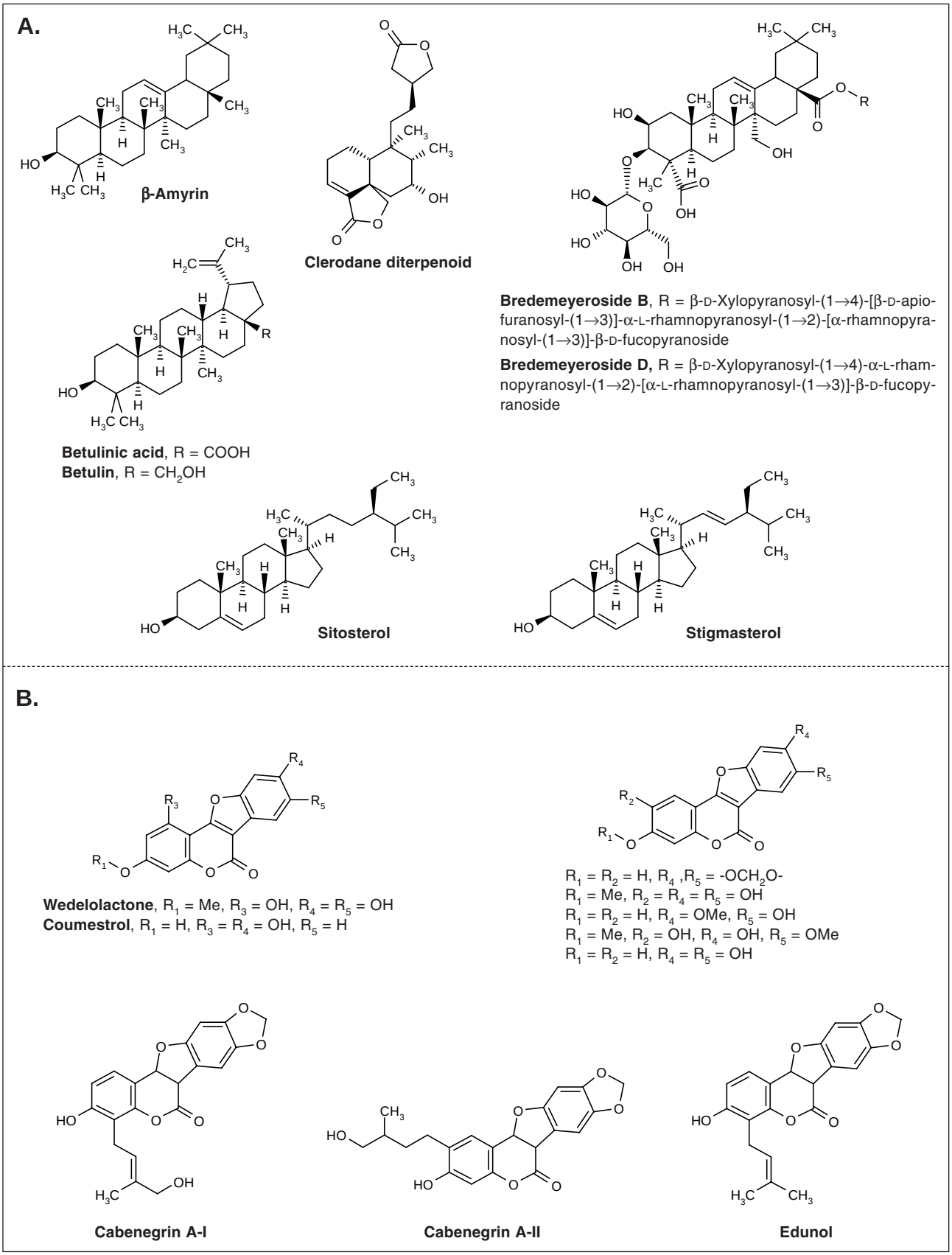

Fig. 5. Continued.

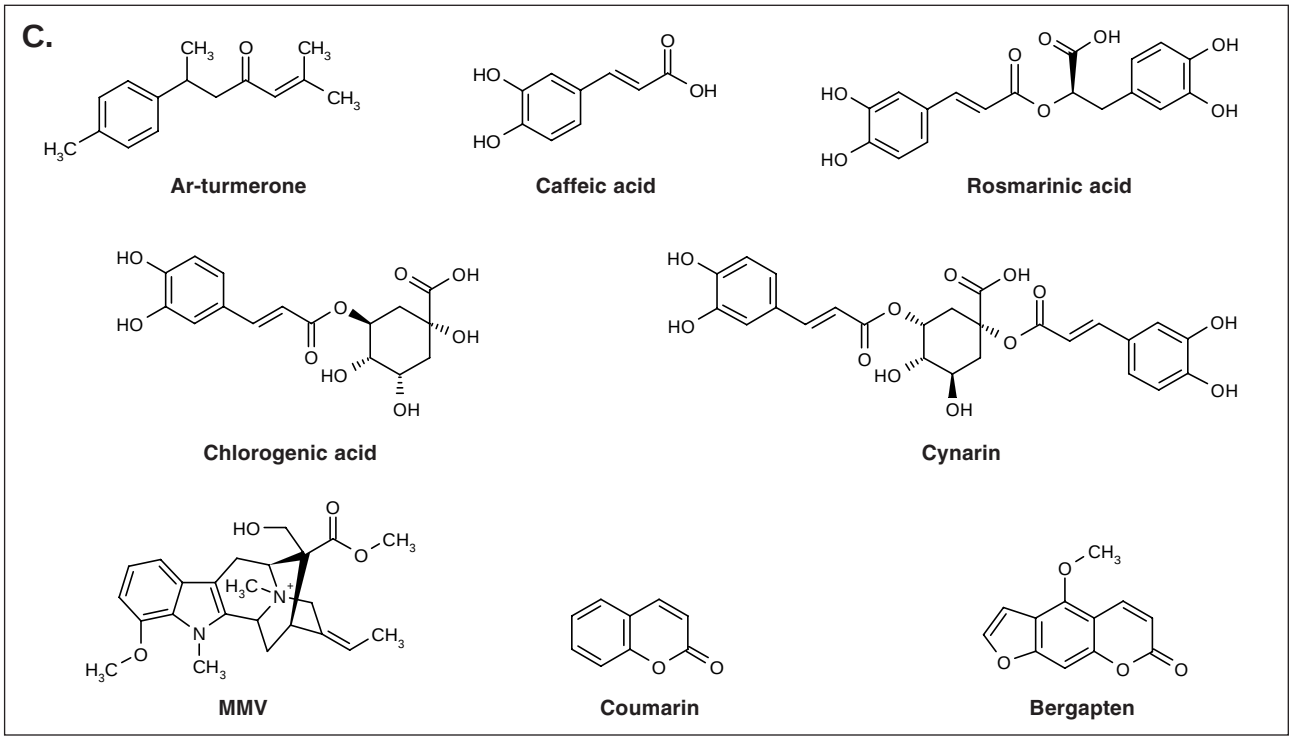

Fig. 5. Chemical compounds isolated from plant extracts with activity against snake venom. **A.** Compounds belonging to the terpenoid and steroid classes. **B.** Coumestans and pterocarpans. **C.** Phenolic compounds.

Among phenolic compounds with antiophidian activity, ar-turmerone, isolated from the hexane extract of *C. longa* roots (Zingiberaceae), popularly known as saffron, was shown to be a potent snake antivenom, able to neutralize both the hemorrhagic effect of *B. jararaca* and the lethal effect of *C. d. terrificus* (28).

Conclusions

Envenomation by snake bite remains a major problem in some countries with tropical and subtropical climates. According to the World Health Organization, it is estimated that 40,000 out of 5 million cases of snake bite are fatal. Serum obtained from horses previously treated with snake venoms is currently the main therapy for envenomation, but such antivenoms must be given immediately and a large percentage of snake bite victims do not have access to conventional treatment, especially in the developing countries. Also, snake bite victims may develop adverse reactions, including anaphylactic shock, and the antivenoms may not always prevent the local effects of envenomation, such as myonecrosis, hemorrhage and edema (69, 70). The use of endogenous plants against snake bite envenomation is therefore worth considering and the search for alternative treatments and the therapeutic use of natural plant products have advanced significantly during recent years (20, 71).

The use of plant extracts and isolated chemical compounds as antidotes for snake venom is not only common in places where they do not have prompt access to serum therapy, but is also used as a supplement or alternative to antivenom therapy. Although there are a number of reports on plants from different geographical areas with the ability to neutralize snake venoms, only in a few cases have the chemical compounds responsible for such activity been identified.

Overall, it can be concluded that many plants popularly considered to be active against snake venoms may have antidotal properties due to the great number of active compounds they contain. The isolated components or their mixtures can complement serum therapy, once the mechanism of action of each has been elucidated. These active principles structurally resemble secondary metabolites and this similarity is the basis for their physiological action. The antivenom activity of these extracts may be due to the presence of enzyme inhibitors, chemical inactivators or immunomodulating principles (72). The efficacy of plant species as inhibitors of the toxic and pharmacological actions of snake venoms may be attributed to the presence of multiple factors. The active substances identified are mostly low-molecular-weight compounds that exhibit more than one biochemical/pharmacological property in addition to the antidotal effect. Further studies on the isolation, structural characterization and mechanism of action of these natural inhibitors must be carried out in the future.

Popular culture is usually wise and can help guide scientific studies. The biotechnological application of these inhibitors as alternative remedies and as supplements to serum therapy, and as important starting points for the synthesis of new therapeutic drugs of medical interest, should therefore undergo further scientific exploration.

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