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Review article

A Review on Venom Enzymes Neutralizing Ability of Secondary Metabolites from Medicinal Plants

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Key Words

anti-venom, medicinal plants, plant constituents, snake venom

Abstract

Objectives: Medicinal plants are vital sources of bioactive compounds that are useful for the treatment of patients with snake bites or are indirectly applicable for boosting the effects of conventional serum therapy. These plants are being used traditionally by local healers and tribes for the treatment of patients with snake bites and therefore can be used as an alternative against snake envenomation. Scientifically, using the secondary metabolites of plants to neutralize venom enzymes has an extra benefit of being based on traditional knowledge; also, the use of such metabolites for the treatment of patients with snake bites is cheaper and the treatment can be started sooner.

Methods: All the available information on various secondary metabolites exhibiting venom neutralizing ability were collected via electronic search (using Google books, Pubmed, SciFinder, Scirus, Google Scholar, and Web of Science) and articles of peer-reviewed journals.

Results: Recent interest in different plant has focused on isolating and identifying of different phytoconstituents that exhibit Phospholipase A2 activity and other venom enzyme neutralizing ability. In this support convincing evidence in experimental animal models are available.

Conclusion: Secondary metabolites are naturally present, have no side effect, are stable for a long time, can be easily stored, and can neutralize a wide range of snake enzymes, such as phospholipase A2, hyaluronidase, protease, L-amino acid oxidase, 5'nucleotidase, etc. The current review presents a compilation of important plant secondary metabolites that are effective against snake venom due to enzyme neutralization.

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1. Introduction

Snake envenomation is a serious medical problem. Snake venoms constitute a rich source of phospholipase A2 (PLA2), B, C, and D enzymes, haemorrhigins, transaminase, hyaluronidase, phosphodiesterase, acetyl cholinesterase, cytolytic and necrotic toxins, etc. [1]. Cardiotoxicity, myotoxicity, pre- or post-synaptic neurotoxicity, edema, hemolysis, hypotension, etc. are some effects caused by snake bites [2]. Recent findings showed that 2.4 million envenomation cases with 85,000 - 125,000 deaths occur annually [3]. In India, approximately 35,000 - 50,000 deaths due to snake bite are thought to occur every year [4], and about 5.4 million native people of different tribal groups are known to depend on medicinal plants for the treatment of snake bites [5]. Recently, increased attention has been given to, and much interest has been shown in, the use of traditional medicines.

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Table 1 List of secondary metabolites with their venom neutralization abilities

S. No.	Secondary Metabolite	Plant Name	Mechanism of Action	Mode of Administration/ Study Level	Ref.
1	AIPLAI	*Azadirachta indica*	Inhibition of PLA2	*In vitro* study	1
2	Aristolochic Acid	*Aristolochia sp.*	Induction of PLA2	Injected into the mouse foot pad	12
3	2-Hydroxy-4-Methoxy Benzoic Acid	*Hemidesmus indicus*	Neutralization of venom hemorrhagic activity	Injected intradermally into mice	31
4	Rosmarinic Acid	*Cordia verbenacea*	Inhibition of myotoxic activity and PLA2	Injected intramuscularly into the right gastrocnemius muscle of mice	16
5	β-sitosterol	*Eclipta prostrata*	Neutralization of enzymes	*In vitro* and *in vivo* study	3,18
6	Quercetin-3-O-α-rhamnoside	*Euphorbia hirta*	Inhibition of phospholipase-A2	*In vitro* study	21
7	8-methoxycoumestrol	*Medicago sativa*	Inhibition of edema, hemorrhage, and cardiotoxicity	Injected intravenously into mice	23
8	Diterpenes	*Baccharis trimera*	Inhibition of edema and PLA2	*In vitro* and *in vivo* study	7

Snake bites require emergent medical care such as serotherapy, which is the only scientifically recognized treatment for this envenomation and in which anti-venom (serum made from the venom of a snake) is used. The first anti-venom against the Indian Cobra was developed in 1895 [6]. However, that anti-venom has certain drawbacks: it may not be readily available in the rural areas where such snake-bite incidents frequently occur, the composition and antigenic reactivity of the venom may vary due to geographic and taxonomic diversities of the snakes, and some patients may suffer from adverse allergic reactions to the anti-venom [7].

Even now, in most of the rural and tribal areas, individuals depend on native herbal medicine systems for their major health care, including treatment for snake bites. Medicinal plants are a source of bioactive pharmacological compounds that can be used directly in the treatment of patients with snake bites or indirectly as supplements to anti-venom immunotherapy. Therefore, medicinal plant extracts can be used as an effective alternative treatment, either alone where antivenom immunotherapy is not carried out promptly or as beneficial supplements [8-10].

Many plant constituents are used in traditional medicine and act against the various effects induced by snake bites. The toxicity caused by the proteins and the enzymes present in snake venom can be neutralized by the natural inhibitors present in these plants [2]. These natural components, such as phenolic compounds, alkaloids, acids, proteins, etc., can be utilized and exploited to create alternative treatments and therapies for snake envenomation [7]. This review summarizes the different reported effects and characteristics of these natural plant components against snake venom.

2. Alkaloids

Atropine, an alkaloid found in the Solanacae family, inhibits the venoms of the black and the green mamba: *Dendroaspis angusticeps* and *D. polylepsis*, respectively. It is a cholinergic blocker that is thought to decrease the effects of the neurotransmitter released at the cholinergic nerve terminals by the above-mentioned snake venoms [1]. *Azadirachta indica* PLA2 Inhibitor (AIPLAI) is extracted from the leaf of *Azadirachta indica* (neem) by using methanol and inhibits the PLA2 enzymes of cobra and viper venoms in a dose-dependent manner [11]. Another alkaloid, aristolochic acid (AA) (8-methoxy-6-nitrophenanthro (3, 4 -d- 1,3-dioxole-5-carboxylic acid), is found in *Aristolochia radix*. The enzymatic and pharmacological activities of a basic PLA2 from *Vipera russelli* venom is inhibited by this alkaloid. However, the disadvantage of AA is that it only restricts the edema-inducing activity; it has no role in recovery [12].

MMV (12-methoxy-4-methyl voachalotine) is extracted from the T*abernaemontana catharinensis* plant and inhibits the lethal effect of the crotoxin found in the venom of the *Crotalus durissus terrificus* snake [13]. Table 1.

3. Acids

Aristolochic acid is mostly found in *Aristolochia sp*. and plays an important role in increasing the immunity to and inhibiting the phospholipases of snake venoms, mainly their lytic and edematose properties [14]. Organic nitrocompounds, such as aristolochic acids and aristolactams, which contain a phenanthrene nucleus, are produced in the roots of the *Aristolochia sp*. The most abundant aristolochic acid I interacts with the edema-causing enzymes of Indian viperidae [15]. Aristolochic acid acts as a noncompetitive inhibitor of the enzyme by forming a 1:1 complex with PLA2 with a resolution of 1.7 Å, which causes a significant change in the secondary structure of the protein [12]. This inhibitor recognizes subtle differences occuring in the isoforms of PLA2 [15]. 2-OH-4-methoxy benzoic acid is isolated from *Hemidesmus indicus* and has active anti-inflammatory, antipyretic, and anti-oxidant activities, as well as potentiated antiserum action, against *Daboia russellii* venom and viper venom. The methoxy and hydroxy functional groups of this compound neutralize the lethal and hemorrhagic activities of the venom [1].

Rosmarinic acid (RA), which is an ester of caffeic acid and 3, 4-dihydroxyphenyllactic acid [2-O-cafeoil-3-(3, 4-dihydroxyphenyl)-R-lactic], was reported for the first time in *Cordia verbenacea*. It has anti-inflammatory and antimyotoxic properties against snake venoms and isolated toxins. The myotoxicities, as well as the edema activities, of the Lys49 PLA2s BthTX-I and the Asp49 isoform BthTX-II of the *Bothrops jararacussu* (*B. jararacussu*) venom are repressed by RA. Circular dichroism results reflect no significant conformation changes between rosmarinic acid and PLA2 [16]. RA from the methanolic extract of *Argusia* (or *Messerschmidia* or *Tournefortia*) *argentea* (Boraginaceae) was reported to inhibit the hemorrhagic activities of the crude venoms of *Trimeresurus flavoviridis*, *Crotalus atrox* (*C. atrox*), *Gloydius blomhoffii*, and Bitis arietans, as well as snake venom metalloproteinases, HT-b (*C. atrox*), bilitoxin-2 (Agkistrodon bilineatus), HF (*B. arietans*), and Ac1-proteinase (*Deinagkistrodon acutus*) [17].

4. Steroids

Steroids form complexes which are held together by van der Waals and hydrophobic forces [7, 14]. One such steroid is sitosterol (β-sitosterol), which occurs freely or in the form of glycosylated sitosterol; its monounsaturated analogue, stigmasterol, is a very profuse type of steroid. Sitosterol and stigmasterol are extracted from plants like *E. prostrata*, *H. ampla*, and *Pluchea indica* and are active against different snake venoms, such as *B. atrox*, *B. jararaca*, *B. jararacussu*, *Crotalus durissus terrificus*, *Daboia russelli*, *Lachesis muta*, and *Naja kaouthia* [3, 18]. β-sitosterol and stigmasterol isolated from *E. prostrate* have been shown to be completely effective against *C. durissus* terrificus venom and to have anti-myotoxic effects against *B. jararaca*, *B. jararacussu*, and *Lachesis muta* snake venom [3]. *Pergularia daemia*, a milk weed of the Asclepiadaceae family, consists of β-sitosterol and shows inhibitory potential against PLA2 and L-amino acid oxidases of *Naja naja* venom. The high affinity binding between β-sitosterol and enzymes was investigated by using molecular docking studies [19].

Cholesterol is another steroid. It is present in onion skins and in the roots of *Ehretia buxifolia Roxb*. Steroidal alkaloids isolated from the ethanolic crude extract of *Solanum campaniforme* have been shown to neutralize the myotoxicity and skin necrosis induced by *Bothrops pauloensis* crude venom, but to have no effect against the venom's PLA2 [20].

5. Flavanoids

Flavanoids are one of the foremost plant components that work against PLA2, lipooxygenase, etc. They possess anti-inflammatory, anti-hepato-toxic, anti-hypertensive, anti-arrhythmic, hypocholesterolemic, anti-allergic, antitumor, and enzyme-inhibiting properties. The flavonoid rutin weakly inhibits the group I PLA2 from *Naja naja* and strongly inhibits the group II PLA2 from *Vipera russelli* and *Crotalus atrox*. Other examples of flavonoids are primetin (5, 8-dihydroxyflavone), a constituent of *Primula denticulata*, and quercetin, a potent inhibitor of lipooxygenase, as well as hesperidin, isoquercitrin, luteolin, kaempferol, and apigenin [2, 7, 14]. Quercetin-3-O-α-rhamnoside is a flavonoid isolated from the plant *Euphorbia hirta* and is known to inhibit the PLA2, hemolytic, and hemorrhage-inducing activities of the *Naja naja* venom when used in a ratio of 1:20 (venom: Quercetin-3-O-α-rhamnoside) w/w. [21]. Morelloflavone, a flavanone- (C-3 C -8")-flavone biflavonoid, is isolated from *Garcinia madruno* extracts and has been reported to inhibit the enzymatic, myotoxic, edema-forming, and anticoagulant activities induced by *C. durissus cumanensis* venom PLA2 [22].

6. Coumestans

Among the coumestans, the most important is wedelolactone, which is active against South American crotalid venoms from *Crotalus durissus terrificus*, *B. jararaca*, *B. jararacussu*, and *Lachesis muta*, as well as the North American crotalids *C. viridis viridis* and *Agkistrodon contortrix*. Wedelolactone, which is isolated from *Eclipta prostrata*, selectively inhibits 5-lipoxygenase. Wedelolactone also exerts several pharmacological actions: anti-myotoxic, antihemorrhagic, anti-proteolytic, and antiphospholipasic activities. Demethylwedelolactone from the same plant has also been identified to have antihepato-toxic constituents [14]. 8-methoxycoumestrol, a product present in very low amounts in *Medicago sativa L*., is prepared as a sodium salt derivative. It mimics wedelolactone by showing antimyotoxic activity and by inhibiting the edema and hemorrhage activities and the cardiotoxicity of *Bothrops jararacussu* crude venom [3, 23].

7. Pterocarpans

Cabenegrins A-I and A-II are two pterocarpans that are isolated from the plant popularly named "*Cabeça-de-negro*" ("negro´s head"). They are used in the Northeast Brazilian folk medicine "*Específico Pessoa*", which is used against snakebite envenomation. The genus *Erythrina* consists of many other bioactive pterocarpans, among which the bark of *E. berteroana* (Leguminosae), an antiophidic medicinal plant in Guatemala, is common. Edunol, another pterocarpans, which is isolated from the root of the *Harpalyce brasiliana* plant found in Northeast Brazil and the Mexican antiophidic plant *Brongniartia podalyrioides*, has been shown to neutralize the lethal actions of the Bothrops jararacussu crude venom and the *Bothrops atrox* venom, respectively. This compound has also been obtained via chemical synthesis, and the synthesized compound has shown anti-myotoxic, anti-proteolytic, and anti PLA2 activities [3, 14, 24].

8. Terpenoids

Among diterpenes, clerodane diterpenoid, Bt-CD, which is also known as 7α-hydroxy-3, 13-clerodadiene-16, 15:18, 19-diolide, is isolated from *Baccharis trimera*. It is an active component containing anti-proteolytic and anti-hemorrhagic properties against snake venoms, especially Bothrops snake venoms. This inhibitor also neutralizes the hemorrhagic, fibrinogenolytic, and caseinolytic activities of class P-I and III metalloproteinases isolated from *B. neuwiedi* and *B. jararacussu* venoms. The edema induced by crude venoms, metalloproteases, and basic and acidic PLA2s was also inhibited by Bt-CD [7].

Among triterpenoids, the pentacyclic triterpenes betulin and betulinic acid are the most common triterpenes, and they are extracted from *Betula alba* and show anti-PLA2 activity. Betulinic acid is a better inhibitor of PLA2. Bredemeyeroside B and Bredemeyeroside D are triterpenoid saponins; they are isolated from *Bredemeyera floribunda* and show anti-lethality activity *against Bothrops jararaca* snake venom [7]. 4-nerolidylcatechol is isolated from *Piper* sp. and exhibits anti-PLA2 and anti-myotoxic effects against purified myotoxins from *Bothrops* sp. venoms [24, 25]. Other examples of pentacyclic triterpenes are oleanolic acid, lupeol, ursolic acid taraxasterol, α-amyrin, β-amyrin, friedelin, epifriedelinol, alnusenone, etc. [14]. Another natural triterpenoid saponin, which is extracted from the root of *Glycyrrhiza glabra* (licorice), is Glycyrrhizin, which has anti-inflammatory activity [26]. A triterpenoid 1-hydroxytetratriacontane-4-one (C34H68O2) is isolated from the methanolic leaf extract of Leucas aspera Linn and has been shown to significantly neutralize the spectacled cobra (*Naja naja naja*) venom, which has been shown to induce lethal activity in a mouse model [27]. The di- and triterpenoids annonalide, humirianthol, acrenol, and lupeol [28] are found in *Humirianthera ampla*, a member of the *Icacinaceae* family; among these, only lupeol partially inhibits the hemorrhage, edema, pro-coagulant, and myotoxic activities caused by *Bothrops* venom [18].

Lupeol acetate, which is isolated from Indian sarsaparilla *Hemidesmus indicus* (*L.*) *R. Br.* (Asclepiadaceae), is active against *Daboia russelli* venom-induced lethality, as well as hemorrhage, defibrinogenation, edema, and PLA2 activities; it also neutralizes the lethality, cardiotoxicity, neurotoxicity, and respiratory changes induced by *Naja kaouthia* venom [3]. Arjunolic acid, another pentacyclic triterpene, is found in the root extract of *Combretum leprosum* and has been shown to reduce the edema, skin hemorrhage, and pro-coagulating effects of *Bothrops jararacussu* snake venom [29].

9. Some Miscellaneous Compounds

Among tannins, persimmon, tannin from the fruit of *Diospyros kaki*, is active against *Laticauda semifasciata* and *Trimeresurus flavoviridis* venoms. Ellagic acid, a tannin isolated from *Casearia sylvestris*, inhibits the venoms of the Bothrops genus [1, 30] Ellagic acid has been reported to be the major constituent in *Euphorbia hirta*, whose methanolic extract inhibits the effects of *Naja naja* venom. Ar-turmerone, a phenolic compound isolated from the *Curcuma longa* (Zingiberaceae) plant, neutralizes the hemorrhaging and lethality caused by *B. jararaca* and *C. d. terrificus* snake venoms [24].

10. Conclusion

The evidence necessary to establish a scientific basis for the use of traditional plants and their constituents to counter the effects of snake venom exists. Thus, use of herbal and medicinal plants and the constituents of those plants with anti-venom activity to treat patients with snake bites may be thought of as a well-established, very effective substitute for conventional serum therapy for snakebite envenomation. However, most of the research on herbal and medicinal plants has been on plants from different regions; thus, the scientific community must find plant constituents that having anti-venom properties.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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