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SNAKE VENOM, ANTI-VENOM AND ROLE OF MEDICINAL PLANTS ACTIVE AGAINST SNAKE ENVENOMATION

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ABSTRACT

Snake bites cause major death and morbidity all across the world, including India. Despite the fact that there are numerous snake species, only a handful of them are potentially harmful to people. Snake antivenom is the only treatment choice for snake bite therapy, although it has several limitations in clinical practise, such as species specificity, difficulties in availability, price, and appropriate storage conditions. The medicinal plants, which are locally available and frequently employed by traditional healers, require special consideration in this regard. A large range of botanicals and active principles have been studied for their pharmacological qualities in the treatment of snake bites. However, other unknown plants that are reported to have a significant part in this issue must be investigated further. Antiserum, on the other hand, does not give adequate protection against venom-induced haemorrhage, necrosis, and nephrotoxicity, and it frequently causes hypersensitivity responses. India has a long history of medicinal plant use. Many Indian medicinal herbs, particularly in rural regions, are described in literature as being used to heal snakebite victims. Only a few species, however, have been professionally researched, and even fewer have had their active components extracted and structurally and functionally defined.

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INTRODUCTION

Poisonous animal bites have been a severe problem in the world from very early times. This is not an exception in India. Snakes, scorpions, spiders, and many more species fall within this group. Snake bites are the most dangerous of them, causing a large number of deaths and morbidities. Snakes are extraordinary creatures that thrive on land, at sea, in forests, meadows, lakes, and deserts. Most snake bites, however, are caused by non-venomous snakes.

Snake envenomation is a major worldwide health concern. The World Health Organization has designated snakebite as a **"Neglected Tropical Disease"**. As a result, this might be seen as a worldwide health risk for individuals in general, and rural populations in poor nations in particular. It is an occupational danger, particularly in agriculture, for farmers, agricultural labourers, villages, migrant populations, and hunters. It is a severe health risk that causes high mortality and significant pain in sufferers. The highest incidence and fatality rates from snake bites have been observed in South and Southeast Asian nations with substantial agricultural operations and a diverse range of snake species[1]. As a result, no accurate study has yet been undertaken on a global scale to quantify the incidence of snake bites. There are around 3000 recognised species of snakes, with approximately 300 of them being venomous. In India, 53 of the 216 species are toxic. It is believed that about 200,000 poisonous bites occur in India each year, with 35,000-50,000 of these being fatal[2]. The figures are arbitrary because the majority of incidents go unreported. In rural regions, where the majority of bites occur, victims are typically sent to traditional healers, who neither record nor document the occurrences, resulting in a scarcity of trustworthy epidemiological data. Poor health services, difficult and inconvenient transportation, incorrect traditional beliefs, and a delay in anti-snake venom medication are the primary causes of increased mortality related with scorpion bite.

Snakes are classified into several families based on their physical traits. Atractaspididae, Elapidae, Hydrophidae, and Viperidae are the families of poisonous snakes. The primary families on the Indian subcontinent include Elapidae, which includes the common cobra, king cobra, and krait, Viperidae, which includes Russell's viper, pit viper, and saw-scaled viper, and Hydrophidae, which includes sea snakes[3]. The majority of toxic species in India include Ophiophagus hannah (king cobra), Naja Naja (common cobra), Daboia rusellii (Russell's viper), Bungarus caeruleus (krait), and Echis carinatae (saw-scaled viper).

Snakebites are a public health risk on the Indian subcontinent, resulting in significant morbidity and mortality[4]. According to conservative estimates, there are one million accidents worldwide each year, resulting in 600,000 injuries and more than 20,000 deaths[5]. Other estimates estimate yearly global occurrences at 5 million, with 40,000 or more fatalities - close to 10% mortality related to malaria[6]. More than 200,000 cases have been documented in India alone, with an estimated 35,000 to 50,000 people dying each year[7].

Antiserum is the sole available treatment agent worldwide. Antivenom is created by immunising animals such as horses, goats, and rabbits with specific snake venom and then isolating the specific immunoglobins from their blood[8-9]. Snake venom contains a complex mixture of enzymatic and hazardous proteins such as phospholipase A2 (PLA2s), myotoxins, hemorrhagic metalloproteinases and other proteolytic enzymes, coagulant components, cardiotoxins, cytotoxins, and neurotoxins[10-12]. For the treatment of snakebite, traditional herbal therapy is widely available in rural regions. Plants are utilised as antidotes for snake envenomation by rural communities in India and other areas of the world, either alone or in combination. Plants are said to be antidotes for snakebites, with a variety of plants reported to be antidotes in traditional medicine[13].

TYPE OF VENOMOUS SNAKE

Ophitoxaemia is a very unique word that describes the clinical spectrum of snake bite envenomation. About 500 of the 2500-3000 snake species found globally are poisonous. Snakes are classified into families based on their physical properties like as scale arrangement, dentition, osteology, mycology, sensory organs, and so on. Atractaspididae, Elapidae, Hydrophidae, and Viperidae are the families of poisonous snakes. The primary families on the Indian subcontinent include Elapidae (common cobra, king cobra, and krait), Viperidae (Russell's viper, pit viper, and saw-scaled viper), and Hydrophidae (sea snakes)[14].Of the 52 dangerous species in India, Ophiophagus Hannah (king cobra), Naja naja (common cobra), Daboia rusellii (Russell's viper), Bungarus caeruleus (krait), and Echis carinatae (saw-scaled viper) account for the bulk of bites and subsequent morbidity[15].

SNAKE BITE

A snake bite is an injury produced by a snake, which frequently results in puncture wounds created by the animal's fangs and, in rare cases, envenomation. Although the majority of snake species are non-venomous and kill their prey by constriction rather than venom, venomous snakes (15% of 3000 known species) are claimed to be found on every continent except Antarctica[16,17].

FREQUENCY OF SNAKE BITE

Every year, between 35,000 to 50,000 individuals are estimated to die from snake bites in India; however, unreported incidence may be higher in rural India. Estimated snake bites and (death) cases were reported[18] as 25,000(30) in Europe, 20,000(100) in the Middle East, 45,000(15) in the United States and Canada, 3,000(5,000) in Central and South America, 10,00,000(20,000) in Africa, 40,00,000(1,00,000) in Asia, and 10,000(200) in Oceania, for a global total of 5 million (1,25,000). Deaths from snake bites are uncommon in Australia, Europe, and North America, but common in Southeast Asia, Southeast Asia, and Sub-Saharan Africa[19]. In Zimbabwe, 4 out of every 5 children under the age of 8 perished in 274 instances studied[20].

IDENTIFICATION OF POISONOUS AND NON-POISONOUS SNAKE

Poisonous snakes generally possess the characters like –

1. Vertically elliptical shaped cat like pupil
2. A small depression (termed pit) between the eyes and nostrils
3. Triangle shaped head e.g. Copperheads and rattle snakes, exception- Elapids
4. Underside scales of tail go completely all the way across in a single row from the anal plate; the very tip of the tail may possess two scale rows
5. Head and body both are seen during swimming time
6. Generally of multiple colors
7. Emitting a warning rattle (a dry, whirring sound) e.g. Rattlesnakes, not to be confused by the sound due to the vibration of several other poisonous and non-poisonous snakes.

In contrast, non-poisonous snakes generally possess the characters like –

1. Round pupil in the centre of eye
2. 'U' shaped head
3. Two rows of scales from the vent to the tail end
4. Only head is seen during swimming time
5. Generally of one colour
6. Mostly stripes are from head to tail

ENVENOMATION

Envenomation is fully voluntary, which means that all poisonous snakes can bite (dry bite) without injecting venom into their victim[21]; around 20% of snake bites are dry bites. The amount of venom released varies greatly across species; for example, the Gaboon viper delivers the most poison per bite of any snake[22].

GENERAL SYMPTOMS OF SNAKE BITE

The severity of snake bites is determined by a variety of factors, including the species and size of the snake, the location of the body bitten, the amount of venom injected, and the victim's age and health. Children are more prone to experience severe symptoms because to their smaller body size and exposure to a higher quantity of venom. Terror and panic are typical following a snake bite and can result in a distinct set of symptoms mediated by the autonomic nervous system. The symptoms of bites from different varieties of snakes vary greatly. Most snake bites, whether venomous or not, have some form of local consequence.Over 90% of the time, there is little discomfort and redness, however this varies depending on the place. Viper and certain cobra bites can be very painful, with local tissue becoming sensitive and badly swollen within 5 minutes. This region may potentially bleed and blister, leading to tissue necrosis in the long run. Lethargy, bleeding, weakness, nausea, and vomiting are some frequent early symptoms of pit viper and viper bites. Over time, symptoms such as hypotension, tachypnea, severe tachycardia, severe internal bleeding, altered sensorium, renal failure, and respiratory failure might emerge.

Despite causing catastrophic harm, bites from the Mojave rattlesnake, kraits, coral snake, and speckled rattlesnake are said to produce little or no discomfort. If bitten by a specific type of rattlesnake, victims may describe a "rubbery," "minty," or "metallic" taste. Spitting cobras and rinkhalses can spit poison into their victims' eyes, causing instant agony, ophthalmoparesis, and, in severe cases, blindness[23,24]. Some Australian elapids and most viper envenomations produce coagulopathy, which can be severe enough to cause spontaneous bleeding from the mouth, nose, and even old, seemingly healed wounds. Internal organs, including the brain and intestines, may bleed, resulting in ecchymosis (bruising) of the victim's skin.

Toxins found in the venom of elapids, such as sea snakes, kraits, cobras, king cobras, mambas, and many Australian species, cause neurotoxicity[25]. The sufferer may exhibit unusual visual abnormalities, such as blurriness. Paresthesia affecting the entire body, as well as difficulties speaking and breathing. The sufferers may die from respiratory failure if they are not treated quickly. Necrosis of muscle tissue is caused by the venom released by several varieties of cobras, practically all vipers, some Australian elapids, and some sea snakes.Muscle tissue throughout the body will begin to die, a disease known as rhabdomyolysis, which can cause kidney damage due to myoglobin build-up in the renal tubules, culminating in hypotension and acute renal failure, finally leading to death.

Dry snakebites, as well as those caused by non-venomous species, can cause significant harm to the victim by inflicting deep puncture wounds and microbiological contaminations, such as Clostridium tetani, which can be found in the snake's saliva and teeth.

SNAKE BITE MANAGEMENT

There are two important aspects of snake bite management -

1. Proper first aid
2. Anti-venom serum therapy

Because rural residents are obliged (because to a lack of effective treatments) to go to adjacent towns and cities for medical assistance, valuable time is squandered in travelling and planning transportation (scenario may be same universally). Proper first-aid utilising herbal formulas can significantly minimise the number of fatalities caused by snake bites.

SNAKEVENOM

Snake venom (yellow, green, or even colourless) is a viscous egg-like liquid primarily composed of toxic protein toxins such as neurotoxins, cardiotoxins, blood clotting toxins, bleeding toxins, and enzymes (>50), as well as other major components such as small peptides, amino acids, carbohydrates, lipids, nucleosides, biological amines, and metal ions; produced in modified parotid glands normally responsible for secreting saliva, stored in structures called alveoli behind the animal's eyes and ejected through its hollow tubular fangs.Fresh snake venom is neutral or weakly acidic, and it becomes alkaline when exposed to air for an extended period of time. When maintained at room temperature for 24 hours, fresh venom generates foam and becomes non-venomous and rancid (toxicity disappear following UV irradiation and heat treatment; dealt with formaldehyde the toxicity also disappear but antigenic property is retained).

SNAKE VENOM - BASIC COMPOSITION

Venom is nothing more than a poisonous snake's secretion, which is manufactured in venom glands. It is modified saliva that contains a variety of various bioactive proteins and polypeptides that an animal uses for defence or to paralyse its prey[26]. Not only is each snake's venom unique, but there is also a minor variation between species, juveniles and adults, and even snakes of the same species but from different geographical locations. Protein accounts for 90-95% of the dry weight of venom. These proteins have the potential to be hazardous or non-toxic. Cytotoxins, cardiotoxins, neurotoxins, and hemotoxins are the different types of venoms. Neurotoxic venom is found in cobras, mambas, sea snakes, kraits, and coral snakes, whereas hemotoxic venom is found in rattle snakes, copper heads, and cotton heads. Some snakes have a mix of neurotoxins and hemotoxins[27].

Snake venom is not made up of a single component, but rather a concoction of hundreds, if not thousands, of various peptides, proteins, enzymes, and compounds. Approximately 20 distinct types of harmful enzymes have been discovered to be present in snake venom in variable combinations and doses. Acetyl-cholinesterases, L-amino acid oxidases, serine proteases, metalloproteinases, and phospholipases-A2 are the most prevalent enzymes found in snake venom. Many non-enzymatic toxins found in snake venom, such as neurotoxic, cardiotoxin, myotoxin, and the three-finger family of proteins, play a key part in venom toxicity.

SNAKE VENOM TYPES

Snake venom is classified as hemolytic or neuropathic. The hemolytic venom is more powerful than the neuropathic venom. The toxins in snake venom are classified as blood circulation toxins (e.g. Viper, Trimersurus stejnegeri, Agkistrodon acutus; symptoms: rapid swelling, bleeding, pain, bite region turns purplish, black, and necrotic, may cause death due to heart failure if not treated effectively within 4 hours), nerve toxins (e.g. Bungarus fasciatus, B. multicinctus; symptoms: bleeding, swelling (e.g. Cobra and King Cobra; nervous symptoms). Snake toxins serve a wide range of purposes. Neurotoxin (Fasciculins: attack cholinergic neurons by destroying acetylcholinesterase, resulting in tetany and death; Dendrotoxin: inhibits neurotransmission by blocking the exchange of (+) and (-) ions across the neuronal membrane, paralysing the nerve, e.g.- Mambas; -neurotoxin: block Ach flow, causing numbness, e.g.- Kra (Phospholipases: enzyme that convert phospholipids molecule to a lysopholipid- causes hole in cell membrane, e.g.-Japanese Habu snakes; Cardiotoxin: muscle venom and prevents muscle contraction, stops heart-beat, e.g.- King Cobra and some other Cobras; Haemotoxin: destroy RBC, slowly progressing venom, e.g.- Vipers and members of Naja genus).

According to Fry[28], snake toxins are formed by the recruitment of genes from the following protein families: acetylcholinesterase, disintegrin/metalloproteinase, AVIT, complement C3, crotasin/beta defensin, cystatin, endothelin, factor V, factor X, kallikrein, kunitz-type proteinase inhibitor, LYNX/SLUR, L-amino oxidase, lectin, natriuretic peptide, betanerve growth factor, phospholipase A(2), SPla/Ryanodine, vascular endothelial growth factor, and whey acidic protein/secretory leuko-proteinase inhibitor. In the evolution of snake venom, toxin recruitment events were discovered to occur 24 times.

Calvete et al.[29] used RP-HPLC, N-terminal sequencing, MALDI-TOF peptide mass fingerprinting, and CID-MS/MS to analyse the protein composition of the venom of the East African Gaboon viper (Bitis gabonica gabonica) and discovered 35 proteins with molecular masses ranging from 7 to 160 kDa belonging to 12 toxin families. Serine proteinases (26.4%), Zn^{2+}-metalloproteinases (22.9%), C-type lectin-like proteins (14.3%), PLA2s (11.4%), and bitiscystatin (9.8%) were the most abundant proteins, while other protein classes, such as bradykinin-potentiating peptides, dimeric disintegrins, Kunitz-type inhibitor, DC fragments, sv-VEGF, CRISP, and L-amino acid oxidase, comprises of about 1.3 and 3.4% of the total venom proteome.

UTILITY OF SNAKE VENOM

Snake venoms are used to treat heart disease, high blood pressure, cancer (contortrostain produced by Agkistrodon contortrix - is cytostatic in nature and has been shown to lower the growth rate of breast cancer in mice), tumour, polio, neurological disorders (enzymes from cobra venom have been shown to cure Parkinson's and Alzheimer's diseases), excessive bleeding (a blood clotting protein in Taipan venom stops bleeding during surgery or after major Other applications for snake venom include the treatment of viruses (because venom contains phospholipidases that tear down cell membranes), the treatment of ageing, and the usage of some in commercial wrinkle cream.

ANTI-VENOM

Calmette (1895) developed anti-venom (specific therapy of envenomation by parenteral injection of horse or sheep derived polyclonal anti-venom) to neutralise venom toxins and was tested against Indian Cobra (Naja naja).

ANTI-VENOM TYPES

Anti-venoms can be classified into monovalent (when they are effective against a given species' venom) or polyvalent (when they are effective against a range of species, or several different species at the same time) types.

GENERIC NAME

Equine (horse derived) / Ovine (sheep derived) immune-globulin F(ab')2 fragments.

PHARMACOTHERAPEUTIC CLASS

i. Immunosera and
ii. Immunoglobulins

ANTI-VENOM SELECTION

The selection of the suitable anti-venom is a critical step. Venom detection kits (available only in Australia and consisting of a quick two-step enzyme immunoassay in which wells are coated with antibodies to the various snake venoms through a swab from the bite site, blood, or urine) aid in determining the kind of anti-venom. Polyvalent anti-venoms are employed when venom type identification is not possible.

LIMITATIONS OF ANTI-VENOM

I. Cause various side effects
II. Can't undo damage already caused by venom, so anti-venom treatment should be started as soon as possible.
III. Mostly administered intravenously but the route may not be uniformly effective
IV. Production is time consuming and expensive
V. Limited supply
VI. Liquid anti-venom may loose its activity due to protein precipitation, if not stored properly
VII. Must be preserved always as freeze-dried ample

SIDE EFFECTS OF ANTI-VENOM

Side effects of anti-venom therapy include anaphylactic reaction (difficulty breathing and swallowing; hives; itching, especially of feet or hands; reddening of skin, especially around ears; swelling of eyes, face, or inside of nose; unusual tiredness or weakness, sudden and severe), serum sickness (enlargement of lymph glands; fever; generalised rash and itching; inflammation of joints), and pyrogen reaction (probably due to the action of high concentrations of non-immunoglobulin proteins present in commercially available hyper-immune anti-venom[30].

ETHNOBOTANICAL FOR THE TREATMENT OF SNAKEBITE

In India, around 54 million indigenous people of diverse ethnic groups live on varied terrains. These indigenous communities have their own distinct culture, religious traditions, eating habits, and extensive understanding of traditional medicine. Even today, indigenous and specific local groups employ herbal medicine to treat a range of ailments, with herbs in particular used as folk medicine to treat snakebites. **"Table - 1"** summarises the many plant species utilised as folk medicine for snake bite treatment. Topical use of plant extracts to affected areas, eating leaves or barks, drinking or injecting extracts can all help to reduce snake venom activity.

TABLE : 1 - TRADITIONAL PLANTS USED AGAINST SNAKEBITE.

SL. NO.	PLANT SPECIES	FAMILY	PARTS USED	DIRECTION	ADMINISTRATION
1.	*Abrus precatorius*	Leguminosae	Roots	Unknown	Oral (5 days)
2.	*Abutilon indicum*	Malvaceae	Leaf, Fruits	Leaf juice mixed with jaggery	Oral (2days)
3.	*Acacia leucophloea*	Mimosaceae	Bark	Bark paste	External (1 Week)
4.	*Acalypa indica*	Euphorbiaceae	Leaf	Paste	External (3–4 days)
5.	*Achyranthes aspera*	Amaranthaceae	Leaf, Stem	Paste	External (3 Weeks)
6.	*Acorus calamus*	Araceae	Rhizome	Paste	External (7 days)
7.	*Angle marmelos*	Rutaceae	Root bark	Water Decoction	Oral (2 Weeks)
8.	*Aerva lanata*	Amaranthaceae	Rhizome	Unknown	Oral (11 days)

9.	*Alangium salvifolium*	Alangiaceae	Root bark	Decoction	Oral(twice a day up to 4 days)
10.	*Allium cepa*	Liliaceae	Skin bulb	Paste	External application (5 days)
11.	*Andrographis paniculata*	Acanthaceae	Whole plant	Decoction, Paste	Internal/External (5–14 days)
12.	*Andrographis lineata*	Acanthaceae	Leaf Flower	Juice	Oral (5 days)
13.	*Argemone mexicana*	Papaveraceae	Leaf Seed	Decoction	Oral (7 days)
14.	*Aristolochia indica*	Aristolochiaceae	Root	Paste	External (1 Week)
15.	*Azadirachta indica*	Meliaceae	Flower	Decoction	Oral (7 days)
16.	*Caesalpinia bonduc*	Caesalpiniaceae	Seeds	Paste	External (2 Weeks)
17.	*Calendula officinalis*	Asteraceae	Flower	Juice	Oral (4 days)
18.	*Calotropis gigantean*	Asclepiadaceae	Root	Paste with ghee	Oral (3–7 days)
19.	*Cassia alata*	Caesalpiniaceae	Leaf	Paste	Oral (21 days
20.	*Cassia tora*	Caesalpiniaceae	Leaf	Decoction	External (14 days)
21.	*Achillea millefolium*	Asteraceae	Whole plant	Paste	Oral (6 days)
22.	*Sapindus emarginatus*	Sapindaceae	Bark	Paste	Oral (5 days)
23.	*Semicarpus anacardium*	Anacardiaceae	Root	Unknown	Oral (7 days)
24.	*Solanum torvum*	Solanaceae	Flower	Paste	External (8 days)
25.	*Strychnos nux-vomica*	Loganiaceae	Stem Bark	Paste	External (12 days)
26.	*Syzygium cumini*	Myrtaceae	Stem Bark	Decoction	Oral (14 days)
27.	*Teprhosia purpurea*	Leguminosae	Root	Decoction	Oral (7 days)
28.	*Thymus vulgaris*	Lamiaceae	Whole plant	Juice	Oral (14 days)
29.	*Terminalia arjuna*	Combretaceae	Bark	Paste	External (5 days)
30.	*Trichodema zeylanicum*	Boraginaceae	Root	Aqueous extract	Oral and External (3 days)
31.	*Tylophora longifolia*	Asclepiadaceae	Leaf Flower	Unknown	Unknown
32.	*Vitex negundo*	Verbenaceae	Leaf	Paste	External (5 days)
33.	*Wedelia calendulae*	Asteraceae	Leaf	Juice	Internally (14 days)
34.	*Citrus limon*	Rutaceae	Ripe skin	Paste	External (3 days)
35.	*Clinacanthus mutans*	Acanthaceae	Leaf	Paste	External (7 days)
36.	*Curcuma longa*	Zingiberaceae	Rhizome	Paste	External (3 Weeks)
37.	*Cymbopogon citrates*	Poaceae	Whole plant	Fresh plant	Repel snakes (Night)
38.	*Cyperus rotundus*	Cyperaceae	Rhizome	Decoction	Oral (7 days)
39.	*Dalbergia melanoxylon*	Fabaceae	Stem bark	Decoction	Oral (6 days)
40.	*Eclipta alba*	Compositae	Whole plant	Paste	Oral (14 days)
41.	*Eclipta prostrata*	Compositae	Leaf	Paste	External (21 days)
42.	*Ehretia buxifolia*	Ehretiaceae	Root	Paste	External (7 days)
43.	*Euphorbia hirta*	Euphorbiaceae	Whole plant	Decoction	Oral (5 days)
44.	Erythrina excelsa	Fabaceae	bark	Juice/paste	Both (3–7 days)
45.	*Feronica limonia*	Rutaceae	Root	Juice	Oral (3 days)

46.	*Gloriosa superba*	Liliaceae	Tuber	Paste	External (2–5 days)
47.	*Gymnema sylvestre*	Asclepiadaceae	Root	Tincture	Oral (4 days)
48.	*Glycine max*	Leguminosae	Seeds	Juice	Oral (Week)
49.	*Helianthus annuus*	Asteraceae	Seed	Oil	External (14 days)
50.	*Hemidesmus indicus*	Asclepiadaceae	Root	Decoction	Oral (7 days)
51.	*Tragia involucrate*	Euphorbiaceae	Whole plant	Juice	Oral (6 days)
52.	*Morus alba*	Moreaceae	Leaf	Juice	Oral (3 Weeks)
53.	*Leucas cephalotes*	Lamiaceae	Leaf	Paste/Juice	Oral (Twice a day for 6 days)
54.	*Madhuca longifoila*	Sapotaceae	Nut	Paste	External (2–3 days)
55.	*Mimosa pudica*	Mimosaceae	Whole plant	Paste	External (5 days)
56.	*Momordica charantia*	Cucurbitaceae	Flower	Paste with olive oil	External (3 days)
57.	*Ocimum sanctum*	Lamiaceae	Leaf	Juice	Oral (8 days)
58.	*Phyllanthus emblica*	Euphorbiaceae	Fruit	Juice	Oral (14 days)
59.	*Piper nigrum*	Piperaceae	Flower	Paste with ghee	Oral (4 days)
60.	*Rauvolfia serpentina*	Apocynaceae	Root	Unknown	External (10 days)

IN-VIVO PLANT EXTRACTS ACTIVITY AGAINST SNAKE VENOM

Natural snake venom inhibitors serve an important role in neutralising the degrading effects of venom toxins. For many years, it has been known that animal sera and some plant extracts are capable of neutralising snake venom. The goal of this study is to highlight contemporary work with natural snake venom inhibitors while also reviewing previous findings, including those discovered in plants. The medicinal importance of these natural inhibitors may lead to the creation of novel therapies for a variety of disorders, as well as the development of effective antivenoms for the treatment of ophidic accidents.

The most recent work with mice for assessing complete crude extracts is described in **"Table - 2" .** The venom dosage is a crucial aspect in determining if the herbal ingredients will have a neutralising effect. Protein precipitation, enzyme activation, chelation, adjuvant action, antioxidant, protein folding, and many more processes are used to neutralise snake venom.

TABLE : 2 - INVESTIGATED PLANT EXTRACTS ACTIVITY AGAINST SNAKE VENOM[31-63]

SL. NO.	SNAKE SPECIES	PLANT (FAMILY)	PART	EXTRACTS
1.	*Viper russelli*	*Acalypha indica* (Euphorbiaceae)	Leaves	Methanol
2.	*Naja naja*	*Alocasia cucullata* (Araceae)	Roots	80% ethanol
3.	*Naja naja, Daboia russelli*	*Andrographis paniculata* (Acanthaceae)	Herb	90% ethanol, Methanol
4.	*Naja nigricotlis*	*Annona senegalensis* (Annonaceae)	Rootbark	Methanol
5.	*Bothrops jaracaca*	*Apuleia leiocarpa* (Leguminosae)	Roots	Water
6.	*Naja naja*	*Aristolochia sp.* (Aristolochiaceae)	Roots	Ether, Methanol
7.	*Bothrops asper*	*Asclepias curassavica* (Apocynaceae)	Leaves	--

8.	*B. jararacussu, B. moojeni, B. alternatus,*	*Scleria pterota* (Cyperaceae)	Leaves	--
9.	*Viper russelli*	*Tamarindus indica* (Leguminosae)	Seed	95% Ethanol
10.	*Bothrops jaracaca*	*Wilbrandia ebracteata* (Cucurbitaceae)	Roots	Water
11.	*Naja naja*	*Withania somnifera* (Solanaceae)	Roots	--
12.	*Daboia/viper russelli*	*Vitis vinifera* (Vitaceae)	Grape Seeds	Methanol
13.	*Bothrops jaracaca*	*Vernonia condensata* (Compositae)	Leaves	Water
14.	*Vipera russellii, Naja kaouthia*	*Vitis negundo* (V erbenaceae)	Roots	Methanol
15.	*Bothrops jaracaca*	*Bredemeyera floribunda* (Polygalaceae)	Roots	Water
16.	*Bothrops atros*	*Brongniartia podalyriaides* (Leguminosae)	Root	Petrol-methylene chloride
17.	*Bothrops jaracaca*	*Brunfelsia unifora* (Solanaceae)	Leaves	Water
18.	*Bothrops asper*	*Buddleja nitida* (Scrophulariaceae)	Leaves	--
19.	*Bothrops jaracaca*	*Casearia sylvestris* (Fiacourtiaceae)	Seeds	Water
20.	*Bothrops asper*	*Cedrela tonduzii* (Meliaceae)	Leaves, stems	--

21.	*Bothrops asper*	*Citharexylum macrodenium* (V erbenaceae)	Leaves	--
22.	*Bothrops asper*	*Croton draco* (Euphorbiaceae)	Stems	--
23.	*Bothrops jaracaca*	*Chiococca brachiata* (Rubiaeeae)	Roots	Water
24.	*Echis ocellatus, Bitis arietans and Naja nigricollis.*	*Crinum jagus* (Amaryllidaceae)	Bulb	Methanol
25.	*Bothrops jaracaca*	*Cynara scolymus* (Compositae)	Leaves	Water
26.	*Echis carinatus*	*Diodia scundens* (Rubiaceae)	Aerial part	95% ethanol
27.	*Laticauda semifasciata*	*Diospyros kaki* (Ebenaceae)	Fruits	Tannin
28.	*Bothrops jaracaca*	*Dorstenia brasiliensis* (Moraceae)	Roots	Water
29.	*Crotaius durissus, Calloselasma rhodostoma*	*Eclipta prostrata* (Asteraceae)	Herb	Ethanol, Butanol
30.	*Echis carinatus*	*Ehretia buxifolia* (Boraginaceae)	Rootbark	Methanol
31.	*Bothrops jaracaca*	*Elephantopus scaber* (Compositae)	Leaves	Water
32.	*Vipera russellii and Naja kaouthia*	*Emblica officinalis* (Euphorbiaceae)	Roots	Methanol

33.	*Vipera sp*.	*Geranium sp.* (Geraniaceae)	Herb	Water
34.	*Bothrops jaracaca*	*Harpalyce brasiliana* (Fabaceae)	Roots	--
35.	*Daboia russellii, Naja kaouthia*	*Hemidesmus indicus* (Asclepiadaceae)	Roots	Methanol
36.	*Echis ocellatus, Naja n. nigricollis*	*Hibiscus aethiopicus* (Malvaceae)	Herb	Water
37.	*Bothrops alternatus*	*Lychnophora pinaster* (Asteraceae)	leaves	Dichloromethane, ethanol
38.	*Bothrops alternatus*	*Mandevilla velutina* (Apocynaceae)	Roots	Water
39.	*Bothrops jaracaca*	*Marsypjanthes hyptoides* (Labiatae)	Herb	Water
40.	*Bothrops jaracaca*	*Mikania glomerata* (Compositae)	Leaves	Water
41.	*Naja naja kaouthia*	*Mimosa pudica* (Mimosaceae)	Herb	Water
42.	*Bothrops jaracaca*	*Morus Alba* (Moraceae)	Stems and leaves	--
43.	*Echis carinatus, Naja hannah*	Mucuna pruriens (Papilioncee)	Seeds	Water
44.	*Bothrops jararacussu* and *Bothrops neuwiedi*	*Musa paradisiaca* (Musaceae)	Stem	Juice
45.	*Naja nigricollis and Echis ocellatus*	Pakia biglobosa (Mimosaceae)	Stembark	Methanol /Water

46.	*Bothrops jaracaca*	Penellia ternate (Araceae)	Rhizome	--
47.	*Bothrops jararacussu*	*Pentaclethra macroloba* (Mimosaceae)	Bark	Water
48.	*Bothrops jaracaca*	*Periandra mediterranea* (Leguminosae)	Roots	Water
49.	*Bothrops jaracaca*	*Periandra pujalu* (Leguminosae)	Roots	Water
50.	*Bothrops asper*	*Persea americana* (Lauraceae)	Seeds	--
51.	*Naja naja*	*Picrasma quassioides* (Simaroubaceae)	Leaves	Water
52.	*Naja melanoleuca, Naja kaouthia*	*Schuanniophyton magnificum* (Rubiaceae)	Rootbark	Water
53.	*Bothrops jaracaca*	*Stachytarpheta dichotoma* (Verbenaceae)	Herb	Water
54.	*E. carinatus*	*Strophanthus sp.* (Apocynaceae)	Leaves	Water
55.	*Bothrops asper*	*Struthanthus orbicularis* (Loranthaceae)	Leaves	Ethanol

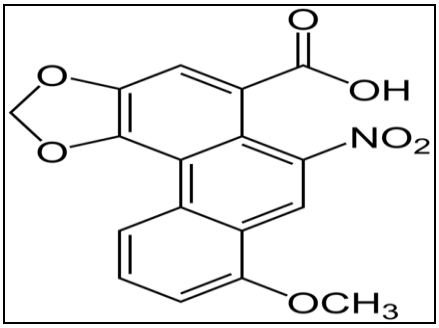
PHYTOCONSTITUENTS ACTIVE AGAINST SNAKE ENVENOMATION

Plant chemicals have played a significant role in the creation of a large number of innovative synthetic medications. **"Table – 3"** lists the compounds identified from plant species that have antiophidian activities**. "Figure - 1"** depicts the phytochemicals that shown effective antisnake venom activities, including plant phenols, alkaloids, triterpenoid, and steroid.

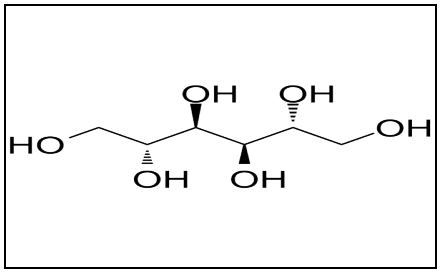
TABLE : 3 - LIST OF ISOLATED CONSTITUENT WITH ANTIOPHIDIAN ACTIVITY[64-83]

SL. NO.	ISOLATED CONSTITUENT	PLANT	ANTIOPHIDIAN ACTIVITY
1.	Anisodamine	*Anisodus tanguticus*	Cholinergic receptor blocking agents
2.	Aristolochic acid	*Aristolochia* sp	Anti- PLA2 activity
3.	Clerodane diterpenoid	*Baccharis trimera*	Anti-proteolytic and anti- hemorrhagic properties

4.	Betulin and betulin acid	*Betula alba*	Anti- PLA2 activity
5.	Bredemeyeroside D	*Bredemeyera floribunda*	Anti-lethal activity
6.	Edunol	*Brongniartia Podalyrloides*	Anti-lethal activity
7.	Rosmarinic acid	*Cordia verbenacea*	Anti- PLA2 activity
8.	Cynarin	*Cynara scolymus*	Anti-lethal activity
9.	Ehretianone	*Ehretia buxifolia*	Anti-lethal activity
10.	Wedelolactone	*Eclipta prostata*	Anti-myotoxic, anti-hemorrhagic activity
11.	Tannins	*Guiera senegalensis*	Anti-lethal activity
12.	2-hydroxy-4-methoxy benzoic acid	*Hemidesmus indicus*	Anti-lethal activity, anti- hemorrhagic activity, coagulant, defibrinogenating, fibrinolytic activity
13.	Edunol	*Harpalyce brasiliana*	Anti-Myotoxicity
14.	D-mannitol, sitosterol	*Mimosa pudica*	Anti-proteolytic, anti-hyalurnidase, antimyotoxicity, anti-lethality
15.	Steroids	*Mandevilla velutina*	Anti-PLA2
16.	4-nerolidylcatechol	*Piper umbellatum, Piper peltatum*	Anti-PLA2, anti-myotoxic
17.	Benzoylsalireposide salireposide	*Symplocos racemosa*	Anti-Phosphodiesterase I
18.	Amide	*Strychnos nux vomica*	Anti-lethal activity, Anti-hemorrhagic, defibrinating, Anti- PLA2 activity
19.	Flavonoids	*Sapindus saponaria*,	Hemorrhagic activity
20.	Caffeic acid and derivatives Chlorogenic acid	*Vernonia condensata*	Antidotes



ARISTOLOCHIC ACID



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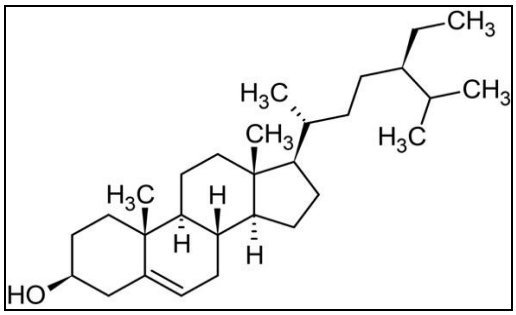
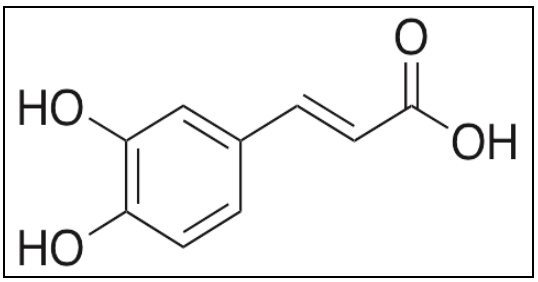
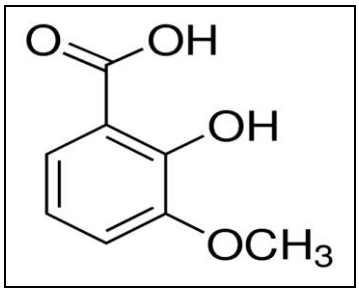


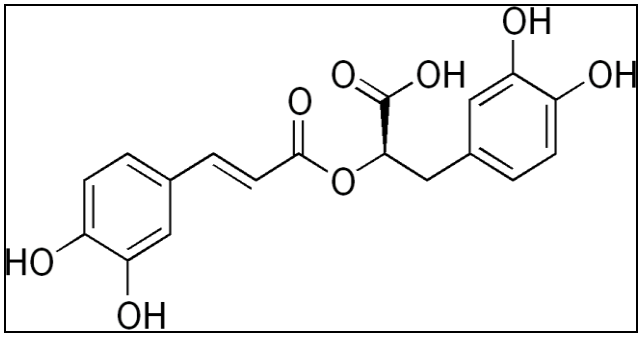
SITOSTEROL



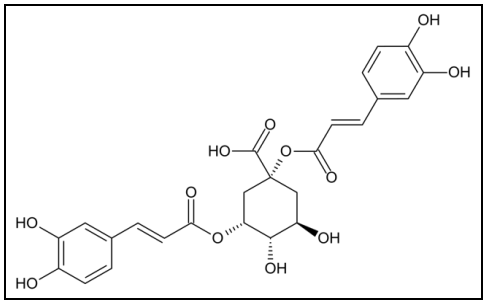
CAFFEIC ACID



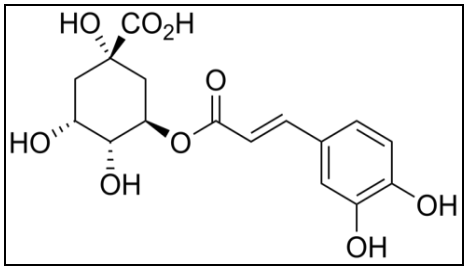
2-HYDROXY-4-METHOXY BENZOIC ACID



ROSMARINIC ACID



CYNARIN



CHLOROGENIC ACID

FIGURE : 1 -PHYTOCONSTITUENTS ACTIVE AGAINST SNAKE ENVENOMATION.

TESTS FOR DETECTION OF SNAKE VENOMS, TOXINS AND VENOM ANTIBODIES

Victims' identification of the snake biting species is frequently difficult, and clinical indications alone are rarely trustworthy due to overlapping symptoms. The detection of snake venom and venom antibodies in bodily fluids is critical in the treatment of snake envenomation. Bioassays, immune-diffiusion, immune-electrophoresis, immune-fluoresence, haemagglutination, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and other methods have been developed for venom detection[84].

ELISA appears to be the best approach for detecting venom as well as venom antibodies[85-87]. The prevalence of cross-reacting venom antigens, as well as the occurrence of several venomous species within a geographical region, makes species identification challenging. During the early 1980s, the lack of particular immunoreagents, poor level sensitivity, lengthy incubation stages, and the necessity for expensive equipment impeded the widespread use of routine diagnostic procedures such as RIA and ELISA. However, great progress has been achieved in the last 10 years toward the development of species-specific ELISA for the detection of venoms/toxins in many regions of the world, notably in poor nations where snake bite is a major medical and social concern. Species-specific immunoreagents for diagnostic applications have been developed using hybridoma technology and affinity chromatography.

CONCLUSION

Snake bite is one of the most common and, at times, lethal phenomena. Herbal plants give a firm platform for the natural therapy of this critical disease, with anti-snake venom being the sole therapeutic alternative accessible but having several downsides. The data shown above clearly show that herbal remedies have a high potential for treating snake bites. Herbal medicinal plants are a significant component of traditional medical systems all throughout the world. Though several of the active plant ingredients are interesting candidates for future antivenom drug molecule development, a single purified chemical may not be enough to entirely negate the harmful effect of snake venom.

Pre-clinical studies to investigate the antivenom effectiveness of appropriate herbal formulations including diverse combinations of these active compounds are thus required. However, before advocating for the safe therapeutic administration of herbal formulations in the clinical care of snake bite patients, the bio-safety and in vivo toxicity of the formulations must be assessed. It is now generally acknowledged that developing herbal medication for snake bite is a challenging process. More research is needed to determine the phytochemicals responsible for these medicinal plants' anti-snake action. The current analysis serves as a foundation for increasing scientists' attention to ethnomedicinally relevant herbs for scorpion bite therapy.

The use of natural medicine has been influenced by the inefficiency of the biomedical health system, as well as its economic effectiveness and cultural acceptability. Snake envenomation is most common in rural India, and medicinal herbs have long been used to treat snakebite. These ethnic groups employ herbal cures against envenomation without antivenom administration, and it is the acknowledged medicine in these areas. Plant extracts are an incredibly rich source of pharmacologically active chemicals and have more than one biochemical/pharmacological activity.

The interaction of such chemicals with toxins/enzymes results in the neutralization/inhibition of their activity. As a result, plant remedies may be effective in the treatment of snakebite and may provide an alternative to antivenom serum.It is suggested that the medicinal plants may be taken for further pharmacological and clinical studies and also recommend for future research.

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CONFLICT OF INTEREST

There is no conflict of interest in this manuscript.

ABBREVIATIONS

PLA_2	- Phospholipase A_2
RP-HPLC	- Reverse phase high performance liquid chromatography
MALDI-TOF	- Matrix assisted laser desorption ionization-time of flight mass spectrometry
CRISP	- Clustered Regularly Interspaced Short Palindromic Repeats of genetic information
VEGF	- Vascular endothelial growth factor
RIA	- Radioimmunoassay
ELISA	- Enzyme-linked immunosorbent assay

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