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Antidotes against snakebite from Ethnobotanical practices of primitive tribes of Tamilnadu

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Abstract

Surveys were carried out during April 2013 to March 2014 to collect information about the plants, which are believed to act as antidotes against venomous snakebite from primitive tribes of Tamil Nadu. Repeated enquiries were made to understand their traditional knowledge, methods of diagnosis and treatment procedures by using medicinal plants. Data were collected about part of the plants which are used, plants vernacular name, their composition, mode of preparation, application, dosages and food restriction during snakebite treatment period. The methanol extracts of the root, stem, bark and leaves of the plants were subjected to different biochemical tests separately for the identification of various active constituents present in plants which are inhibiting the action of snake venom. It was found that a total of 35 plant species belongs to 26 families were used as antidotes against snakebite. Among the tribes whom were interviewed, Kurumba provided more information with regard to medicinal plants and its usage against snake bite.

Keywords: antidotes, secondary metabolites, snakebite, tribal, tribal medicine.

INTRODUCTION

Snakebite is a serious medical, social and economic problem in many parts of the world (Brunda and Sashidhar, 2007). Snake bite, till date remains a public health hazard in tropical countries, especially in India (Gomes *et al*., 2010). India has the highest number of deaths due to snake bites in the world, and according to World Health Organization 35,000-50,000 people are dying every year (Alirol *et al*., 2010). Among the various states in India, high incidences of snakebite happen in Maharashtra followed by West Bengal, Uttar Pradesh, Tamil Nadu and Kerala (Pillay, 2008 and Vijayaraghaven, 2008). Majority of snakebite deaths in India is mainly due to four species, collectively called 'The Big Four' also described as the 'medically important venomous snakes such as the spectacled Cobra (*Naja naja*), Common Krait *(Bungarus caeruleus*), Russell's viper (*Daboia russelli*) and saw-scaled viper (*Echis carinatus*). These Big Four are found throughout India and occur in a wide variety of habitats ranging from forests to country side including desert areas, arid tracts and even well-populated towns, army barracks and Cantonments. They frequent in cultivated areas and are often found in and around human habitations (Sukumaran *et al*., 2012). These species are responsible for thousands of deaths every year (Whitaker and Captain, 2008). The venom of each species of snake is unique with a different composition of toxic and non toxic constituents. Venoms are at least 90% proteins and these are mostly enzymes and about 25 such enzymes have been recorded, the number of which may differ according to species. The snake venom contains enzymes and strongly basic polypeptides. Though snake venoms are often characterized as either neurotoxic (damaging or destroying nerve tissues) and those that produce paralysis, impair the nervous system and death by respiratory shock. It blocks nerve impulses from being carried to the muscles leading to progressive paralysis, stoppage of breathing and stoppage of heart (e.g cobras and kraits).

Haemotoxic (attacking tissue and blood) those that have haemorrhagic effects; cause heavy bleeding internally and externally, traditionally associated with Russell's viper and saw scaled viper).

Envenomation due to snake bites is commonly treated with the administration of horse or sheep-derived polyconal antivenin aimed at neutralization of toxins. A polyvalent anti snake venom is available now a days. This polyvalent is effective for all "Big 4" venomous snake bite (Whitaker and Captain, 2008). Anti venoms are costly and may be with limited supply. Anti venom should be given as quickly as possible, so that the venoms side effects can be managed. It is not widely distributed in rural areas; a very remote area which is far away from town, a bitten person could not able to reach a hospital with in the golden hour (Bawaskar and Bawaskar, 2001). The success of anti snake venom therapy depends on the ability of immunoglobulins that bind and eliminate toxins present in the body (Sharma *et al*., 2002 and Sharma *et al*., 2005). Moreover, Indian anti snake venom was produced using venoms from snakes captured in a tiny geographic area of the state of Tamil Nadu and may therefore be less effective in other regions (Simpson and Norris, 2007).

From time immemorial, herbals have played an important role throughout the world in treating and preventing variety of diseases (Principe *et al*., 1991). Traditional herbal medicine is readily available in tribal areas for the treatment of snakebite. Application of the

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plant or its sap onto the bitten area, chewing leaves and bark or drinking plant extracts or decoctions are some procedures intended to counteract snake venom activity. Plants are used either single or in combination, as antidotes for snake envenomation by rural population in India and in many parts of the world (Samy *et al*., 2008). The curative and preventive snakebite treatments, beliefs and practices are found around the world among different ethnic groups (Owuor *et al*., 2005;

Owuor and Kisangau, 2006). This traditional knowledge is passed on through oral from one generation to the other, and practiced as trial and error methods (Sinha, 1996). Among the Indian states, the South Indian tribes are blessed with rich biological diversity of plants and a high degree of traditional knowledge about medicinal plants and their uses (Natarajan *et al.,* 2013). Many quantitative and qualitative field surveys have documented the detailed utility of specific plants for many aboriginal groups such as Kadars, Kanikars, Irulars, Malasars, Malamalasars, Malaiyalis, Paliyars, Todas and Kotas (Umapriya *et al*., 2011).

Very little research had been done on the claims of herbal medicine as an antidote for snake venom. Thus, the study of herbal antidotes against snake venom is of great importance in the management of snakebite. So, there is an urgency and scope for extensive work in this field to know the modus operandi of herbals which are used as antidotes against snakebite and to preserve and save the traditional knowledge and the value of tribal medicines for our future generations. Hence, this present study was conducted to find out the herbals which are used as antidotes against snakebite in rural areas and herbal constituents which are active against snake bite.

MATERIALS AND METHODS

In order to assess the plants usage against snake bite by the primitive tribes, preliminary surveys were carried out in Kothagiri, Gudalur, Namakkal, Sirumalai, Thamarakkarai, Srivilliputhur and Boluvampatti areas of Tamil Nadu from April 2013 to March 2014. Elderly people and medicine man of the tribal communities such as irulars, kurumbar, malasars, paliyars and paniyars were interviewed. The anti-snakebite plants were identified with the help of them who had very long acquaintance with the usage of herbals against snakebite. Plant species identification was done with the help of standard manuals (Gamble and Fischer, 1950; Mathew, 1983). Village old mothers, 'vaidhyars' or traditional healers, tribal head and house wives were also consulted for specific part of the plants which are used against snakebite (whole plant, root or rhizome, leaf, stem, seed, etc.), vernacular name, their composition (fresh or dried and mixture of other plant used as ingredients), mode of preparation (decoction, paste, powder and juice) mode of application (internally or externally), dosage and food restrictions during period of treatment were carefully recorded in the field note book. Repeated enquiries were made to understand their knowledge, methods of diagnosis and treatment procedures against venomous snakebite. A short description of the plants, their habit, habitat, family, synonyms, local name and other vital parameters were also collected during the field work.

Qualitative phytochemical analysis (Harborne, 1998)

***Preparation of extracts*:** The methanol extract of the plants were subjected to different chemical tests separately for the identification of various active constituents against snake bite. Fresh leaves and bark were homogenized for 5mts in methanol. The homogenized extract was evaporated at 40°C, condensed up to the volume of 1/10, acidified with 2mts sulphuric acid and extracted with chloroform. The chloroform and acid layers were separated and the aqueous acid layers were basified with ammonium hydroxide to pH 10. This was further extracted with chloroform and methanol in the ratio of 3:1 twice and then extracted with chloroform.

Alkaloid test

a. Wagner's test

Preparation of Wagner's Reagent: Iodine 1.27g and potassium iodide was dissolved in 5ml of water and made up to 100ml with distilled water. To the 1ml of the extract, 2ml of Wagner's reagent was added, the formation of reddish brown precipitate indicates the presence for alkaloids.

b. Mayer's test

Preparation of Mayer's reagent: Mercuric chloride (1.3589g) dissolved in 60ml of water and potassium iodide (5.09g) was dissolved in 10ml of water. The two solutions were mixed and made up to 100ml with water. To 1ml of the extract 2ml of Mayer's reagent was added, a dull white precipitate reveals the presence of alkaloids.

Test for Phenolic compounds and Tannins

c. Ferric Chloride Test

The extract 50ml was dissolved in 5ml of distilled water. To this, few drops of natural 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

1. Anthraquinones

0.5ml of the extract was boiled with 10% Hcl for 2 minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate.

2 drops of 10% NH_3 were added to the mixture and heated. Formation of rose-pink colour indicates the presence of anthraquinones.

2. Glycosides

The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. Two drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

3. Reducing Sugars

2ml of the extract was shaken with distilled water and filtered. The filtrate was boiled with two drops of Fehling's solution A and B for a minute. An orange red precipitate indicates the presence of reducing sugars.

4. Saponins

0.2ml of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

5. Flavonoids

0.2ml extract of was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

6. Phlobatanins

0.5ml of the extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate showed the presence of phlobatanins.

7. Steroids

2ml of acetic anhydride was added to 0.5g of the extract of each with 2ml of H_2SO_4. The colour changed from violet to blue or green samples indicates the presence of steroids.

8. Terpenoids

The extract was treated with 2 drops of acetic anhydride, boiled and cooled. Then concentrated sulphuric acid was added from the side of the test tube, brown ring was formed at the junction of two layers and the upper layer turns green which shows the presence of steroids and formation of deep red colour indicates the presence of triterpenoids.

RESULTS

During the survey period, a total of 35 plant species belonged to 26 families and categorized into five groups were recorded that are believed to be used as antidotes against snakebite by 7 different tribal communities in Tamil Nadu. The plant species are arranged in alphabetical order and family wise, vernacular name and parts which are used as antidotes against snakebite are given in (Tab. 1). Detailed information regarding plants' usages are given in the tables 2, 3, 4, 5, 6 and 7. Kurumba tribes had considerable knowledge about plants that are used as antidotes against snakebite in Tamil Nadu. They are using 17 herbals (36.17 %) against venomous snakebite. The tribes such as Irulas, Paliyar and Paniyas are using 7 plants (14.89%), Valiyar and Malasar are using 4 plants (8.51%) and Kattunayakans use only one plant against snake bite (Tab. 2 and Fig. 1). Among the 35 plant species which are used as antidotes against snakebite by tribes of Tamil Nadu, 18 herbs (51.42%) were the main source of medicines followed by climbers 8 (22.85 %), 6 trees (17.14 %) 2 shrubs (5.71 %) and one grass species (2.85 %) (Table 3 and Fig. 2). Within the plant parts used against snake bite, majority of the tribes were using root and rhizome (22 cases) followed by leaf in (15 cases) stem bark in (6 cases) whole plant in (5 cases) seeds in (4 cases) bulbs in (3 cases) and fruits in (2 cases) (Table 4 and Fig. 3). During the study period it was noticed that majority of the tribes used the drugs against snakebite as paste 40 (75.47 %), followed by juice 9 (16. 98%), decoction 3 (5.66 %) and powder 1 (1.88%) (Table 5). In most of the cases, the paste was applied on the bitten site and also internally in the form of juice, decoction and powder. The heavily envenomed snakebite cases, the plant and its parts were made into powder and applied (externally) in bitten site and also given internally (Table 6).

During the survey period, a total of 20 persons (20 -30 age group) were contacted for interview, but nobody shared and provided information about the plants which are used as antidotes against snakebite. 55 persons belonged to the age group of 30-40 were interviewed. Among them, 5 persons provided information on anti snakebite plants and 30 persons belonged to the age group of 40-50 were contacted for interview, a total of 33 persons provided information. A total of 45 persons with the age of above 50 were interviewed, and among them 40 persons had good knowledge and provided more information about anti-snakebite plants and their various uses (Table. 7). It was evident that elderly people had utilized more number of plants against snakebite when compared to younger generation. Women of most of the tribal community had little knowledge of medicinal plants and their uses against snakebite.

Qualitative phytochemical analysis

Phytochemical analyses were performed on a representative sample of nine plants and its parts such as root, whole plant, leaves and rhizome which are most commonly used as antidote against snakebite by the tribals of Tamil Nadu. These plants include: *Abrus precatorius* (Family: Fabaceae), *Aerva lanata* (Family: Amaranthaceae), *Aristolochia indica* (Family: Aristolochioceae), *Azardirachta indica* (Family: Meliaceae), *Curcuma longa* (Family: Zingiberaceae),

Table 1. Medicinal plants used by tribes against snakebite

S. No	Plants species	Family	Vernacular Name	Parts used
1	*Abrus precatorius*	Fabaceae	Kundumani	Seed and root
2	*Aerva lanata*	Amaranthaceae	Pula poo	Leaf and rhizome
3	*Achyranthes aspera*	Amaranthaceae	Nayuruvi	Whole plant
4	*Ageratum racemosus*	Asteraceae	Chattavari	Root
5	*Allium sativum*	Alliaceae	Valli-vangayam	Bulbs
6	*Andrographis paniculata*	Acantheceae	Siriyanangai	Root, leaf and Whole plant
7	*Aristolochia indica*	Aristolochiaceae	Karudakodi	Leaf and Root
8	*Azadirachta indica*	Meliaceae	Veppamaram	Leaf
9	*Brassica juncea*	Brassicaceae	Kaduku	Seed
10	*Calotropis gigantea*	Asclepiadaceae	Erukala	Leaf and Root
11	*Centella asiatica*	Apiaceae	Vellarai	Root
12	*Citoria ternatea*	Fabaceae	Sangu poo	Leaf and root
13	*Citrus limon*	Rutaceae	Yalumachai	Fruit
14	*Curculigo orchiodes*	Hypoxidaceae	Nelapani	Rhizome
15	*Curcuma longa*	Zingiberaceae	Manchal	Rhizome
16	*Cynodon dactylon*	Poaceae	Arugampull	Root
17	*Hemidesmus indicus*	Asclepiadaceae	Nannari	Root
18	*Jasminum auriculatum*	Oleaceae	Kattumulli	Root
19	*Lebelia nicotianiifolia*	Lobeliaceae	Kattupugaielai	Leaf
20	*Leucas aspera*	Lamiaceae	Thumbai	Leaf
21	*Mimosa pudica*	Mimosaceae	Thottachinungi	Whole part and Root
22	*Momordica charantia*	Cucurbitaceae	Pavakkai	Root
23	*Momordica dioica*	Cucurbitaceae	Kattupavakkai	Root
24	*Murraya paniculata*	Rutaceae	Chedichi	Bark
25	*Musa paradisiaca*	Musaceae	Vaalai	Bark
26	*Pergularia daemia*	Asclepiadaceae	Veeliparuthi	Leaf
27	*Phyllanthus emblica*	Euphorbiaceae	Nellikaai	Fruit
28	*Piper betle*	Piperaceae	Vetrilai	Leaf
29	*Piper nigrum*	Piperaceae	Milagu	Seed
30	*Rauvofia serpentine*	Apocynaceae	Serpagandhi	Root
31	*Rhinacanthus nasutus*	Acantheceae	Nagamali	Leaf and Root
32	*Sansevieria roxburghiana*	Agavaceae	Marul or Paampukalli	Bark and Rhizome
33	*Solanum nigrum*	Solanaceae	Manathakali	Leaf and Root
34	*Terminalia chebula*	Combretaceae	Kadukai	Seed
35	*Urginea indica*	Liliaceae	Kattupoondu	Bulb

Table 2. No. of plants used as antidotes against snake bite by various tribes

S. No	Tribal community	No. of plant used as antidotes against snakebite	% wise data
1	Kurumbar	17	36.17
2	Paniyar	7	14.89
3	Paliyar	7	14.89
4	Irular	7	14.89
5	Malasar	4	8.51
6	Valiyar	4	8.51
7	Kattunayakan	1	2.12

Table 3. Categories of plants used as antidotes against venomous snakebite

S. No	Category of plants	No. of species	% wise data
1	Herb	18	51.42
2	Climber	8	22.85
3	Tree	6	17.14
4	Shrub	2	5.71
5	Grass	1	2.85

Table 6. Application of plants for treatment of snake bite

S.No	Applied method	No. of preparations	% wise data
1	External	30	56.60 %
2	Internal	12	22.64 %
3	Both	11	20.75 %

Figure 1. Diagram showing plants used as antidotes against snake bite by tribes

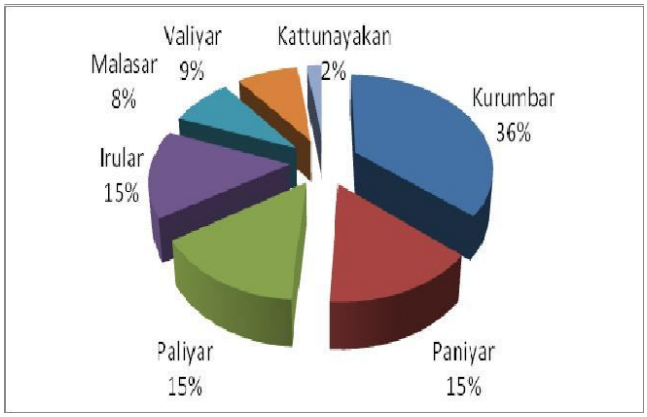

Figure 2. Diagram showing comparative data of plants used against snakebite

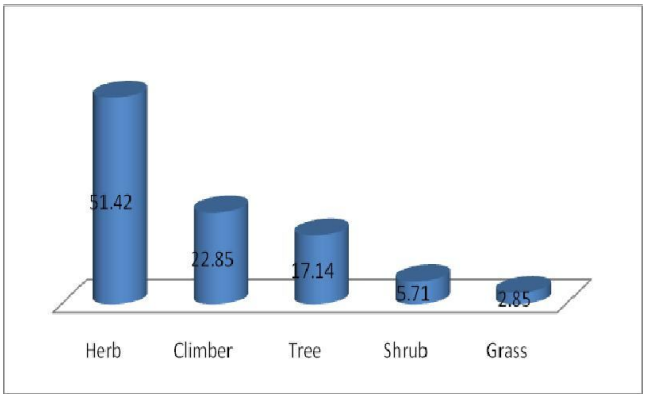

Figure 3. Diagram showing number of prescriptions of different plants parts used in the treatment

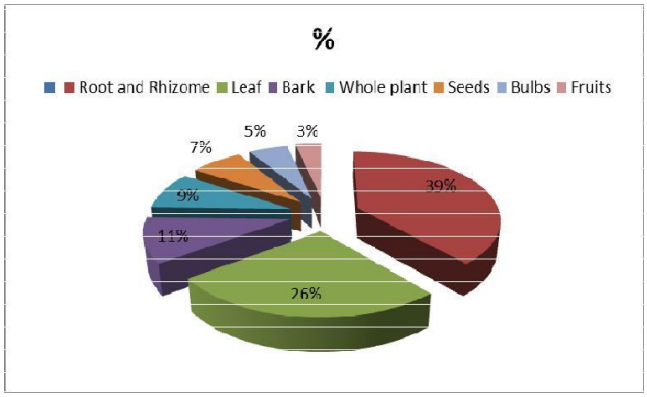

Table 4. Plants parts used by tribes against snakebite

S. No	Plant parts	No. of prescriptions	% wise data
1	Root and Rhizome	22	38.59 %
2	Leaf	15	26.31 %
3	Bark	6	10.52 %
4	Whole plant	5	8.77 %
5	Seeds	4	7.01 %
6	Bulbs	3	5.26 %
7	Fruits	2	3.50 %

Table 5. Preparation of different plant parts used for snake bite treatment

S.No	Mode of preparations	No. of prescription	% wise data
1	Paste	40	75.47 %
2	Juice	9	16.98 %
3	Decoction	3	5.66 %
4	Powder	1	1.88 %

Table 7. Age wise data on tribes

S.No	Age class of tribes	Number of person interviewed	Number of person provided information	% wise data
1	20-30	20	-	-
2	30-40	55	5	6.84 %
3	40-50	30	33	34.24%
4	above 50	45	40	54.79%

Table 8. Result of Qualitative phytochemical analysis

S.No	Plant species	Family	Plant partsused	Alkaloids	Flavonoids	Tannins	Terpenoids	Saponins	Glycosides
1	*Aerva lanata*	Amaranthaceae	Rhizome	-	-	-	-	+	-
2	*Abrus precatorius*	Fabaceae	Root	+	-	-	-	-	-
3	*Aristolochia indica*	Aristolochiaceae	Root	-	-	-	+	-	-
4	*Azardirachta indica*	Meliaceae	Leaf	-	+	-	-	-	-
5	*Curcuma longa*	Zingiberaceae	Rhizome	+	-	-	-	-	-
6	*Hemidesmus indicus*	Asclepiadaceae	Root	-	+	-	-	-	+
7	*Leucas aspera*	Laminaceae	Leaf	-	-	-	+	-	-
8	*Mimosa pudica*	Fabaceae	Whole plant	+	-	-	-	-	-
9	*Sansevieria roxburghiana*	Asparagaceae	Rhizome	-	-	-	-	-	+

+ Indicates presence phytochemicals - indicates absence of phytochemicals

Hemidesmus indicus, Leucas aspera (Family: Lamiaceae), *Mimosa pudica*

(Family: Fabaceae) and *Sansevieria roxburghiana* (Family: Agavaceae). A portion of the aqueous extract that was subjected to phytochemical screening was used for identification of the major secondary metabolites which are believed to inhibit snake venom and its harmful effects. Phytochemical analysis showed that the secondary metabolites were found in the following combinations: Triterpenoids (*Aristolochia indica* and *Leucas aspera)* Flavonoids (*Azardirachta indica),* Alkaloids (*Abrus precatorius, Curcuma longa* and *Mimosa pudica*), and Glycosides (*Hemidesmus indicus* and *Sansevieria roxburghiana)* and Saponins (*Aerva lanata*) (Table 8).

DISCUSSION

This study provides 35 species of antidotes plants and their uses against snakebite. Among these plants, some plant species were previously reported against snake bite (Prasad *et al*., 1987; Karupusamy, 2009; Samy *et al*., 2008; Rajan and Sethuraman, 2008; Shanmugam *et al*., 2009; Revathi and Parimelazhagan, 2010; Murugesan *et al*., 2005; Alagesaboopathi, 2011; Jain *et al*.,2011). Eight species of medicinal plants such as *Ageratum racemosus, Brassica juncea, Centella asiatica, Cynodon dactylon, Lebelia nicotianiifolia, Momordica dioica, , Terminalia chebula* and *Urginea indica* were used for various ailments (Prajapati *et al*., 2003; Rajan and Sethuraman, 2008; Ramya *et al*., 2008; Karuppusamy, 2009; Gomes *et al*.,2010; Ayyanar *et al*., 2011). But no mention has been made about the antidotes activities of these plants against snakebite. However, in the present study it has been recorded that Kurumbas and Paniyar are using these plants against snakebite also.

The roots/rhizomes and leaves are the two major plant parts which are frequently used by the tribes against snakebite cases (Panghal *et al*., 2010). Roots /rhizome are used mostly because they contain high concentration of bioactive compounds such as alkaloids, phenols, steroids, saponins and glycosides. The leaves remain green and available in plenty mostly throughout the year, which are active in photosynthesis and participate in the production of secondary metabolites (Ghorbani, 2005). The use of leaves in the preparation of remedies is also common elsewhere (Ayyanar and Ignacimuthu, 2011). Combination of plant species and use of water, cow milk or goat milk for diluting/mixing various ingredients/formulations have been practiced during the period of treatment.

Elderly people provided more information regarding the medicinal plants than the younger generation. This finding very well agreed with earlier studies (Panghal *et al*., 2010). Some elderly tribal women had little knowledge about medicinal plants and in most cases this traditional knowledge has been kept as a secret. The traditional knowledge is freely transferred within the family especially to male generation. The traditional knowledge has not been documented properly and mainly passed from generation to generation through oral communication. This traditional knowledge is dwindling rapidly in the recent years, because the younger generation changed their mind set to modern lifestyle, modern agricultural practices, cultural changes within the community, rapid shift towards the allopathic medicine. Housing, construction and other developmental activities caused destruction habitat loss and habitat fragmentation, which led to loss of medicinal plants and the traditional knowledge. Similarly threat to traditional knowledge has also been reported from other parts of the country (Panghal *et al*., 2010).

The present investigation proved that all theses nine species of plants such as *Aerva lanata, Abrus precatorius, Aristolochia indica, Azardirachta indica, Curcuma longa, Hemidesmus indicus, Leucas aspera, Mimosa pudica* and *Sansevieria roxburghiana* had considerable amount of secondary metabolites which possess snake venom neutralizing potential. Several chemical constituents like alkaloids, flavonoids, glycosides, saponins and triterpenoids are found in varying proportions in the plants. The anti-snake venom activity has also been previously reported (Cherdchu and Karlsson, 1983; Alam *et al*., 2004; Borges *et al*., 2005; Samy *et al*., 2008; Meenatchisundaram *et al*., 2009). All these classes of chemical compounds are capable of interacting with macromolecular targets (enzymes or receptors) and can more effectively inhibit the toxic effect of snake venoms *in vitro* than *in vivo* (Borges *et al*., 2005). Thus it is obvious that these plants possess potent snake venom neutralizing capacity.

CONCLUSION

This study deals with the anti snake bite plants used by various tribes of Tamil Nadu. The study revealed that tribal people have authentic knowledge on antidotes plants against snakebite based on their ancient culture and ethnic practices. This study brought to light the immense hidden knowledge of tribes about medicinal plants which are used against venomous snake bites.

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