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The anti-snake venom activity of the leaves of *Symplocos cochinchinensis* (Lour.) S.Moore ssp. *laurina* (Symplocaceae)

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Abstract

The in vitro and in vivo anti-snake venom activities of various extracts of the leaves of ***Symplocos cochinchinensis*** *(Lour.) S.Moore ssp.* ***laurina*** *(Symplocaceae) were studied against Russell's viper venom. The in vitro study was carried out by the inhibition of the in vitro Human RBC lysis and the in vivo study was carried out by using Swiss albino mice in modifying the lethal effect of the test dose of the Russell's viper venom. In in vivo model the effectiveness of the extract was evaluated by oral administration of two different doses (200 and 400mg/kg) of the methanolic extract of the leaves of Symplocos cochinchinensis 5 minutes prior to the injection of the venom. The extract markedly decreased the percentage of mortality in venom induced toxicity in mice at the dose of 400mg/kg b.w which indicates the significant anti-snake venom activity of the plant there by justifying its use in the indigenous system of medicine.*

Key words: *In vitro* and *in vivo* anti-snake venom activity, *Symplocos cochinchinensis,* methanolic extract, Russell's viper venom

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INTRODUCTION

Deaths due to snake venomation have been a major health problem since time immemorial. Over the years many medications have been tried to treat snake bites. Antiserum being the only effective therapeutic agent, its development from animal source is time consuming and expensive and also it often produces side effects. Early administration of adequate amounts of snake venom antiserum followed by supportive treatment is the best way of treating envenomation. This necessitates the search for antidotes from herbal sources without these side effects. Although, use of plants against the effects of snake bites has been long recognized , more scientific attention has been given since last 20 years[1] . In almost any part of the world, where venomous snake occurs, numerous plant species are used as folk medicine to treat snake bite[2]. These plants or their extracts or their decoctions or chewing leaves or topical application of its

sap on to the bite area are some important procedures to counteract snake venom activity. In most cases the efficiency of this treatment regimen is unproven compared to the numerous folklore claims, the pharmacological and clinical investigations done on this same are meager[3].

Symplocos cochinchinensis (Lour.) S.Moore ssp. ***laurina*** (Symplocaceae) otherwise known as kabli-vetti or Lodh tree is widely distributed in tropical, subtropical areas in Asia, and America. It is traditionally used in the treatment of various disorders like leprosy, liver disorders, tumors, diarrhea, dysentery, menorrhagia, snake bite, inflammation and uterine disorders[4]. However, no work has been reported on the anti snake venom properties of this plant. Keeping this in view the present study has been undertaken to investigate the *in vitro* and *in vivo* anti snake venom activities of various extracts of the leaves of ***Symplocos cochinchinensis*** *(*Lour).

MATERIALS AND METHODS

2.1 Plant Materials

The fresh healthy leaves of *Symplocos cochinchinensis (*Lour) were collected from Nilgiri hills. It was authenticated by Botanical survey of India and Prof. P. Jayaraman, Botanist and Director, PARC, Chennai. A voucher specimen was deposited in the department for future reference. The leaves were shade dried and coarsely powdered. The powder was then extracted successively with n-hexane, chloroform, ethyl acetate and methanol in soxhlet apparatus. The extracts were dried under reduced pressure using rotary vacuum evaporator. All the four extracts were used to study the *in vitro* anti-snake venom activity and the methanolic extract (200, 400mg/kg) was used to evaluate the *in vivo* anti snake-venom activity.

2.2 Snake venom

Lyophilized snake venom of Russell's viper *(Daboia russelli)* was obtained from CSIR Centre for Biochemical's, New Delhi and preserved at 4^0C. The snake venom was dissolved in 0.9%(w/v) saline, centrifuged and the supernatant was used whenever required. The venom concentration was expressed in terms of dry weight (mg/ml) of the stock venom.

2.3 Animals

Swiss albino mice weighing between 20-25g were used for the study. They were maintained under standard environmental condition (temperature 25-28^0C and 12h light/dark cycle) and they were allowed with standard laboratory feed and water *ad libitum*. The animals were given a week's time to get acclimatized with the laboratory condition. Ethical clearance for the use of animals was obtained from the committee constituted for the purpose**.**

2.4 *In vitro* anti-snake venom activity[5,6]

In vitro anti-snake venom activity was assessed by inhibition of *in vitro* HRBC lysis. In this study n-Hexane, chloroform, ethyl acetate and methanolic extracts of the leaves of *Symplocos cochichinensis* (Lour) were used. The hypo saline induced haemolysis was evaluated *in-vitro* by the method of Roelofsen et al and Balu etal. This method was modified in present study by venom- induced haemolysis.

Blood was collected from healthy human volunteers by vein puncture and heparin was used as an anti coagulant. The collected blood was washed three times with physiological saline solution to make a stock solution of 100mcg/ml. 1 ml of the venom , 1 ml phosphate buffer(pH 7.4) and 1 ml of 1% HRBC were taken in various tubes. Different concentrations of n-Hexane, chloroform,

ethyl acetate and methanolic extracts of the leaves of *Symplocos cochinchinensis* (Lour) Viz., 100, 250, 500 and 1000mcg/ml were added. The extracts were prepared using saline and CMC as suspending agent and the control were prepared omitting the extracts. The anti-snake venom serum(100mcg/ml) was used as standard.

These mixtures were incubated at 37^0C for 30 min and then centrifuged at 1000 rpm for 3 min. The absorbance of the supernatant was measured at 540 nm using spectrophotometer. The percentage inhibition of haemolysis was calculated.

2.5 Acute toxicity studies[7]

Acute toxicity study was carried out using Acute Toxic Class Method as described in **OECD [Organization of Economic Co-operation and Development]** Guidelines No.423.

2.6 *In vivo* anti- snake venom activity[8]

Oral administration of extract 5 min prior to the injection of venom

Thirty adult Swiss albino mice of both sexes were divided into five groups of six mice each. The control group was injected with only venom (lethal dose 61mcg/20 g of the mice , i.p), while the other groups were treated separately with venom, after 5 min of oral administration of anti snake venom serum (10mg/kg) and methanolic extract(200, 400mg/kg), respectively. The mice were observed for 24 hours for the number of mice which were survived.

RESULTS AND DISCUSSION

Plant extract

All the extracts were dried under rotary vacuum evaporator and the extractive values were calculated. The methanolic extract of leaves of *Symplocos chochinchinensis* (Lour) was brownish green thick solid mass and the extractive value was found to be 8.8%9w/w.

Acute toxicity studies

Acute toxicity studies were done by OECD guidelines 423. The methanolic extract was safe and no death was recorded in the mice treated orally with varying doses (250-2000mg/kg) of the extract.

The LD_{50} value of the Russell's viper venom was already reported in the literature 61mcg/20 g of the mice[9].

***In vitro* anti snake venom activity**

In vitro anti-snake venom activity for n-hexane, chloroform, ethyl acetaste and methanol extracts were carried out by HRBC membrane stabilization method. The methanol extract showed significant percentage inhibition(65.9%) when compared to standard which showed 89.5% and the results were tabulated [Tableno.1].

***In vivo* anti-snake venom activity**

In vivo anti snake venom activity of the methanolic extract was studied at two different dose levels of 200 and 400mg/kg body weight respectively. In control group, which was treated with only venom (61mcg/20 g) 33.33% of the mice survived. Mice treated with 200 and 400 mg/kg of extract recorded 66 and 83% survival [Table no.2].

Table No.1 *In vitro* anti-snake venom activity
(Inhibition of human red blood cell lysis method)

S.No	Treatment	Conc.(mcg/ml)	% inhibition
1.	Contorl	-	-
2.	Snake venom antiserum	100	89.5
2.	n-Hexane extract	1000 500 250 100	11.9 8.9 5.8 3.8
3.	Chloroform extract	1000 500 250 100	17.6 13.8 10.5 8.0
4.	Ethyl acetate extract	1000 500 250 100	**59.5** 56.0 44.3 34.3
5.	Methanol extract	1000 500 250 100	65.9 48.1 41.4 32.4

Table No.2 *In vivo* anti-snake venom activity of the methanolic extract of the leaves of *Symplocos cochinchinensis*

S.No	Treatment	Dose(mg/kg b.w)	% Survival
1.	Control	-	33.3
2.	Snake venom antiserum	10	100
3.	Methanol extract	200	66.66
4.	Methanol extract	400	83.33

DISCUSSION

In vitro anti-snake venom activity was carried out by HRBC membrane stabilization method Haemolysis is one of the major cause of snake venomation. Most of the snake venom contains phospholipase and haemolysin[10] , which act on membrane associated phospholipids to liberate lysolecithin. Lysolecitihin acts on the membrane of HRBC causing haemolysis[11]. In the present investigation n-Hexane, chloroform, ethyl acetate and methanol extracts of the leaves of *Symplocos cochinchinensis* at the concentration of 100, 250, 500 and 1000mcg/ml were used to evaluate the activity. These extracts inhibit the haemolysis induced by Russell's viper venom in a concentration dependent manner. The percentage inhibition of haemolysis activity was found to be significant in methanol extract at a concentration of 1000mcg/ml showed 69.5% protection against venom induced haemolysis may be due to the stabilization of the protein in the membrane of HRBC[12]. Hence, it may be suggested that the extract may interact with Russell's viper venom and stabilize the protein in the membrane.

The methanol extract was screened for *in-vivo* anti snake venom activity. The extract at 400mg/kg increased the percentage survival which was comparable to that of standard anti

venom serum. It was observed that the survival of the mice increased progressively with increasing the dose of the extract in a dose dependant manner. The snake venom acts at peripheral neuromuscular junction either post synaptically by binding competitively with the acetylcholine receptor or presynaptically by preventing the release of acetylcholine transmitter from the nerve terminals. The probable mechanism of preventing the neurotoxic effect by *Symplocos cochinchinensis* (Lour) may be by interfering with the acetylcholine receptor sites i.e. by antagonising the actions of the neurotoxic substances in the venom at the acetylcholine receptor sites[13].

CONCLUSION

Incidents of snake bite leading to death are common in many tropical countries. Early administration of adequate amount horse anti snake venom serum followed by supportive treatment is the best way of treating snake venomation. This snake venom anti serum contains horse immunoglobulin's which frequently cause complicated medicated side effects and other proteins cause serum sickness and occasionally anaphylactic shock. Thus, the study of herbal antidotes against snake venom is of great importance. In the present study various extracts of the leaves of *Symplocos cochinchinensis* (Lour) were checked for *in vitro* anti-snake venom activity by inhibiting HRBC lysis. The methanolic extract showed significant inhibitory activity was used for *in vivo* anti-snake venom activity. It showed significant anti-snake venom activity at the dose level of 400mg/kg body weight which was comparable with that of the standard. Thus, the present study has confirmed the ethnomedical use of the plant for the treatment of snake bite. It is hoped that subsequent fractionation of the extract to obtain the pure active compound will enhance its anti-snake venom potential.

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