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In vitro models for determination of anti-snake venom potential of *Erinocarpus nimmonii* and *Hibiscus punctatus*

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Abstract
The present study aims to study the anti-snake venom activities of the local plants, which are native to the western ghats of India. These plants were found to be used by traditional healers in Maharashtra, India to treat patients bitten by snakes. The freeze-dried snake venom powder of *Daboia russelli* was obtained from Hindustan Snake Park, Kolkata. The *Erinocarpus nimmonii* and *Hibiscus punctatus* was identified and authenticated by botanist. Extraction of leaves of both plant by hot extraction method and further evaluated for *In vitro* antivnom activity using three different types of research models, *Viz*. phospholipase A_2 activity, procoagulant activity and Fibrinolytic activity. Plant extracts concentration 0.11 to 0.13 mg for *Erinocarpus nimmonii* and 0.14 to 0.17mg for *Hibiscus punctatus* was inhibited PLA_2 dependent hemolysis, 1.5 to 1.8 mg for *Erinocarpus nimmonii* and 1.6 to 1.9 mg for *Hibiscus punctatus* of plant extracts have shown neutralizing effects in coagulant activity and 0.11 to 0.18 mg for *Erinocarpus nimmonii* and 0.13 to 0.18 mg for *Hibiscus punctatus* showed fibrinolytic activity induced by *Daboia russelli* venom.

Keywords: phospholipase a_2 activity, procoagulant activity, fibrinolytic activity, *In-vitro* antivenom activity, *Daboia russelli* venom

Introduction
Snake chomp stays a general medical condition in numerous nations despite the fact that; it is challenging to be exact with regards to the genuine number of cases. It is assessed that the genuine occurrence of snake envenomation could surpass 5 million every year. Around 1, 00, 000 of these foster extreme screech. The worldwide difference in the epidemiological information reflects varieties in wellbeing detailing exactness as well as the variety of monetary and natural circumstances. Precise records to decide the specific the study of disease transmission or even mortality of snake chomp cases are by and large inaccessible. Clinic records miss the mark concerning the real number, attributable to reliance on conventional healers and specialists of black magic, particularly in agricultural nations. It has been accounted for that in most non-industrial nations, up to 80% of people nibbled by snakes initially counsel conventional experts prior to visiting a clinical focus. Attributable to the postponement, a few casualties kick the bucket during excursion to the clinic [1-3].
Because of significant expense of emergency clinic treatment and inaccessibility of serums venoms, most frequently the provincial individuals find it more helpful to counsel local specialists who are acclaimed for restoring snakebite patients. This proof shows that plant cures utilized by the local specialists are powerful, and there has all the earmarks of being a high pace of endurance among snakebite patients progressed clinical phases of venom harmfulness [1-5]. The present study aims to study the anti-snake venom activities of the local plants, which are native to the western ghats of India. These plants were found to be used by traditional healers in Maharashtra, India.

Materials and Method
Venom Procurement
Snake venom powder in the Lyophilized form was obtained from Hindustan Snake Park, Kolkata and was stored at 4°C.

Medicinal Plants and Preparation of Extracts
Based on escalated writing overview; *Erinocarpus nimmonii* and *Hibiscus punctatus* were chose for utilized as anti-venom activity in present examination. The writing survey demonstrated that the chose plants have potential as anti-venom activity. The *Erinocarpus nimmonii* Grah. Was identified and authenticated by Dr. T. Chakraborty, Scientist D, Joint Director, Botanical Survey of India, Koregaon Park, Pune, India and *Hibiscus punctatus* was identified and authenticated by Dr. R. B. Thoke, Head of Department, Botany, Yashwantrao Chavan Institute of Science, Satara. Both the voucher specimens were deposited at the respective Institutes. *Erinocarpus nimmonii* Grah: voucher specimen sample number is ERINTA-2; *Hibiscus punctatus* Dalz: voucher

specimen sample number is HPUNCT-1. The certificates for the authentication were obtained. The leaves powder of *Erinocarpus nimmonii and Hibiscus punctatus* of plant was beat and the powdered material (250 g) was removed autonomously with petrol ether, chloroform, ethanol (90%) and water using hot extraction system. Directly following ousting the biomass stores by filtration, pooled eliminates were centered around turning vacuum evaporator. The concentrates were also dried using oven at 80°C except for water eliminate. The water remove was dried using shower dryer. Finally completely dried concentrates were checked.

***In vitro* anti-venom Activity**

Phospholipase Activity

Phospholipase A2 action was estimated utilizing an aberrant hemolytic measure on agarose-erythrocyte-egg yolk gel plate by the strategies depicted by Gutierrez *et al*., 1988. Expanding portions of *Daboia russelli* venom (µg) were added to 3mm wells in agarose gels (0.8% in PBS, pH 8.1) containing 1.2% sheep erythrocytes, 1.2% egg yolk as a wellspring of lecithin and 10mM $CaCl_2$. Slides were hatched at 37°C short-term and the breadths of the hemolytic coronas were estimated.

Control wells contained 15µl of saline. The base aberrant hemolytic portion (MIHD) compares to a dose of venom, which created a hemolytic corona of 11mm diameter. [6] The adequacy of antidote (plant extricates) in killing the phospholipase not entirely set in stone by blending consistent measure of venom (µg) with different measure of plant removes (µl) and hatched for 30 minutes at 37°C. Then, at that point, aliquots of 10µl of combinations were added to wells in agarose-egg yolksheep erythrocyte gels. Control tests contain venom without plant separates. Plates were brooded at 37°C for 20 h. Balance was communicated as the proportion of mg antibodies/mg venom which could diminish the distance across of the hemolytic radiance by half when contrasted with the impact prompted by venom alone. [6, 7]

Procoagulant Activity

Procoagulant action was examined by the technique portrayed by Theakston and Reid, 1983 as changed by Laing *et al*., 1992. Different measures of venom broke up in 100µl PBS (pH 7.2) was added to human citrated plasma at 37°C. Coagulation time was recorded and the base coagulant dose [10] not set in stone as the venom portion, which prompted thickening of plasma inside 60 seconds. Plasma hatched with PBS alone filled in as control. In balance measures consistent measure of venom was blended in with different weakenings of plant separates. The combinations were hatched for 30 minutes at 37°C. Then, at that point, 0.1ml of combination was added to 0.3ml of citrated plasma and the coagulating times recorded. In control tubes plasma was brooded with either venom alone or plant extricates alone. Balance was communicated as powerful portion (ED), characterized as the proportion µl antibody (plant extricates)/mg venom at which the coagulating time expanded multiple times when contrasted and thickening season of plasma hatched with two MCD of venom alone. [8]

Fibrinolytic Activity

A changed plaque test was utilized. The base fibrinolytic focus was characterized as the centralization of venom that incited a fibrinolytic radiance of 10mm breadth. Balance tests were performed by hatching a steady measure of venom with different measures of plant removes at 37°C for 1h. After hatching, the blend was applied to the wells in the plaque. After 18h of hatching at 37°C, fibrinolytic coronas were estimated. [9]

Result and Discussion

Phospholipase Activity

In phospholipase movement (PLA2), *Daboia russelli* venom ready to deliver hemolytic haloes in agarose-sheep erythrocytes gels. Around 10µg of Russell's snake venom delivered 11mm breadth hemolytic corona, which is viewed as 1U (U/10µg). This shows that *Daboia russelli* venoms have the chemicals (PLA2) that can lyse sheep Rbc's. Plant removes were fit for restraining PLA2 subordinate hemolysis of sheep RBC's initiated by *Daboia russelli* venom in a portion subordinate way. We found that that 0.14 to 0.17mg for *Erinocarpus nimmonii* and 0.11 to 0.17mg for *Hibiscus punctatus* plant extracts were able to completely inhibit PLA_2 dependent hemolysis of sheep RBC's induced by *Daboia russelli* venom.

Table 1: Phospholipase activity of *Daboia russelli* venom and its neutralization by plant extracts

Plant extracts	Dose of *Daboia russelli* venom (µg)	Neutralization of venom by plant extracts (ED_{50} in mg)
PEEEN	10 (1 Unit)	0.13
CEEN		0.11
EEEN		0.11
AEEN		0.14
PEEHP		0.14
CEHP		0.11
EEHP		0.12
AEHP		0.17

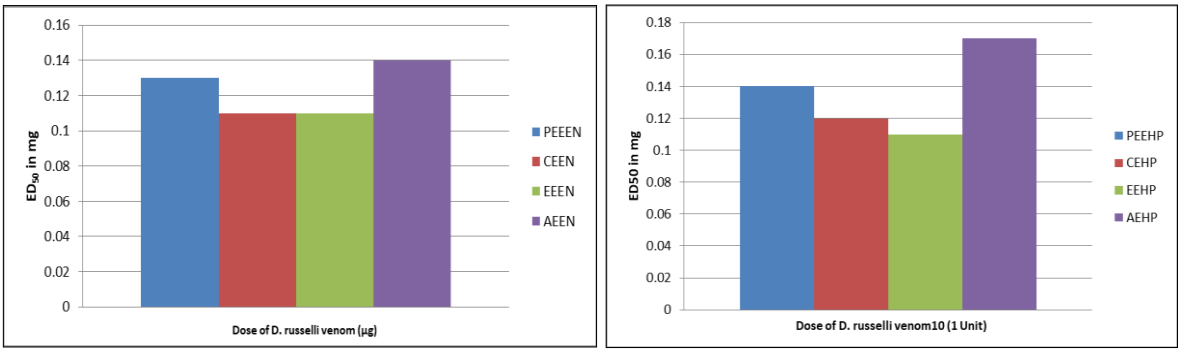

Fig 1: Phospholipase activity of *Daboia russelli* venom and its neutralization by plant extracts

Procoagulant Activity

The base coagulant portion still up in the air as the venom portion actuating thickening of plasma in 60s. Around 120µg of Russell's snake venom thickened human citrated plasma inside 60s. In the balance measure, the shortfall of clump arrangement shows the killing capacity of both plant separates. We viewed that as 1.6 to 1.9 mg for *Erinocarpus nimmonii* and 1.4 to 1.9 for *Hibiscus punctatus* plant extricates had the option to totally kill coagulant movement. High portion of venom caused quick thickening that expected exceptionally high portion of antidote to kill.

Table 2: Procoagulant activity of *Daboia russelli* venom and its neutralization by plant extracts

Plant extracts	Dose of *Daboia russelli* venom (µg)	Neutralization of venom by plant extracts (ED_{50} in mg)
PEEEN	16 ($2LD_{50}$)	1.6
CEEN		1.7
EEEN		1.8
AEEN		1.5
PEEHP		1.6
CEHP		1.8
EEHP		1.4
AEHP		1.9

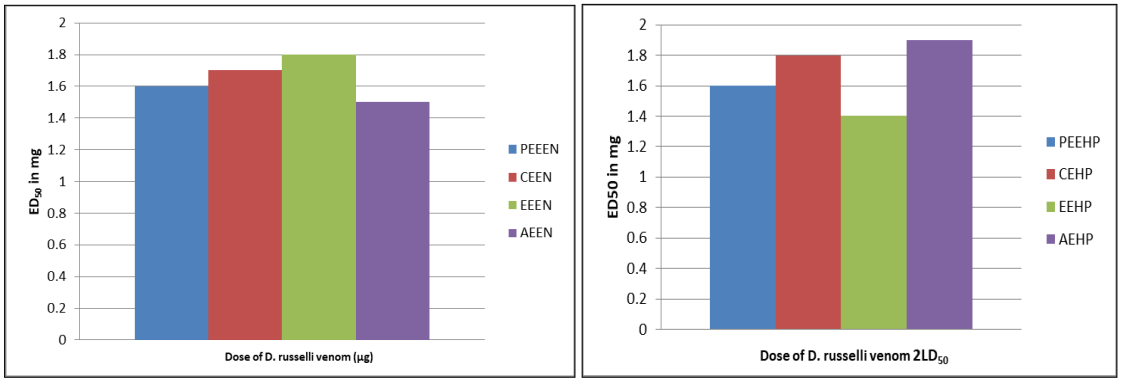

Fig 2: Procoagulant activity of *Daboia russelli* venom and its neutralization by plant extracts

Fibrinolytic Activity

The plant extracts were capable of inhibiting fibrinolytic induced by *Daboia russelli* venom. We found that that 0.11 to 0.18 mg for *Erinocarpus nimmonii* and 0.13 to 0.18 mg for *Hibiscus punctatus* plant extracts were able to completely inhibit fibrinolytic activity (modified plaque assay) induced by *Daboia russelli* venom.

Table 3: Fibrinolytic activity of *Daboia russelli* venom and its neutralization by plant extracts

Plant extracts	Dose of *Daboia russelli* venom (µg)	Neutralization of venom by plant extracts (ED_{50} in mg)
PEEEN	16 ($2LD_{50}$)	0.16
CEEN		0.11
EEEN		0.18
AEEN		0.16
PEEHP		0.17
CEHP		0.18
EEHP		0.13
AEHP		0.15

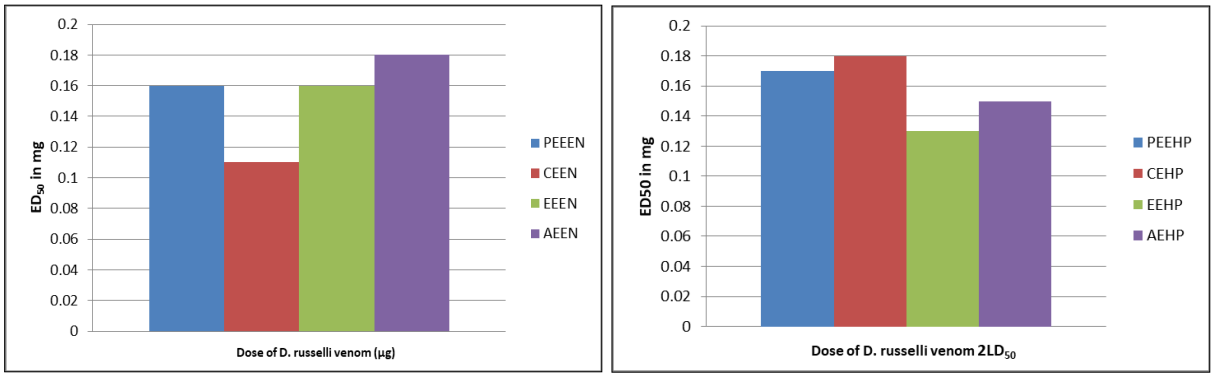

Fig 3: Fibrinolytic activity of *Daboia russelli* venom and its neutralization by plant extracts

Summary and Conclusion

Plant extricates were equipped for repressing PLA2 subordinate hemolysis of sheep RBC's instigated by *Daboia russelli* venom in a portion subordinate way. We viewed that as that 0.11 to 0.13 mg for *Erinocarpus nimmonii* and 0.14 to 0.17mg for *Hibiscus punctatus* plant separates had the option to totally hinder PLA2 subordinate hemolysis of sheep RBC's instigated by *Daboia russelli* venom. The least coagulant portion not entirely settled as the venom portion inciting coagulating of plasma in 60s. We viewed that as 1.5 to 1.8 mg for *Erinocarpus nimmonii* and 1.6 to 1.9 mg for *Hibiscus punctatus* of plant separates had the option to totally kill coagulant action. High portion of venom caused quick thickening that necessary exceptionally high portion of antidote to kill. The plant separates were fit for repressing fibrinolytic induced by *Daboia russelli* venom. We viewed that as that 0.11 to 0.18 mg for *Erinocarpus nimmonii* and 0.13 to 0.18 mg for *Hibiscus punctatus* of plant separates had the option to totally restrain fibrinolytic action (altered plaque measure) prompted by *Daboia russelli* venom.

References

1. Hasson SS. “Anti-snake venom activity of *Hibiscus aethiopicus* L. against *Echis ocellatus* and *Naja n. nigricolis*” Toxicon, 42, 629-642.
2. Meenatchisundaram S. “Studies on antivenom activity of *Andrographis paniculata* and *Aristolochia indica* plant extracts against *Daboia russelli* venom by *in vivo* and *In vitro* methods”, IJST , 2(4): 76-79.
3. Meenatchisundaram S. “Antivenom activity of *Mimosa pudica* root extracts against *Naja naja* and *Bungarus caerulus* venoms”, BDPS, 105-109.
4. Meenatchisundaram S. “Anti-venom activity of medicinal plants- A mini review” Ethnobotanical leaflets, 12, 1218-1220.
5. Rafael Stuani Floriano. “Effect of *Mikania glomerata* (Asteraceae) leaf extract combinedwith anti-venom serum on experimental *Crotalus durissus*(Squamata: Viperidae) envenomation in rats,” IJTB, 57(4), 929-937.
6. Ticli FK, Hage LI, Cambraia RS, Pereira PS, Magro AJ, Fontes MR *et al*. Rosmarinic acid, a new snake venom phospholipase A2 inhibitor from Cordia verbenacea (Boraginaceae): antiserum action potentiation and molecular interaction. Toxicon,2005:46(3):318-327.
7. Manoj Lakhotia, Dinesh Kothari, Dharma Ram Choudhary, Sanjeev Sharma, Pravesh Jain. A case of saw scale viper snakebite presenting as pleuro-pericardial haemorrhage. JIACM,2002:3(4):392-394.
8. Rojas G, Guturrez JM, Gene JM, Gomez M, Cerdas L. Neutralization de las actividades toxicas Y enzimaticas de cuatro venenos de rerpipentes de Guatemala Y Honduras por el antivenenopolivalente producido en Costa Rica. Revista de Biologia Tropical,1987:35:59-67.
9. Laing GD, Theakston RDG, Leite RP, Dias Da Silva WD, Warrell DA. Comparison of the potency of three Brazilian Bothrops antivenoms using *in-vivo* rodent and in-vitro assays. Toxicon,1992:30(10):1219-1225.