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# *In vitro* models for determination of anti-snake venom potential of *Helicteres isora* and *Bixa orellana*

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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 *Helicteres isora* and *Bixa orellana* 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 0.10 to 0.14 mg for *Helicteres isora* and 0.14 to 0.18mg for *Bixa orellana* was inhibited $PLA_2$ dependent hemolysis, 1.5 to 1.8 mg for *Helicteres isora* and 1.6 to 1.9 mg for *Bixa orellana* of plant extracts have shown neutralizing effects in coagulant activity and 0.10 to 0.18 mg for *Helicteres isora* and 0.14 to 0.18 mg for *Bixa orellana* 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
Despite the fact that it is difficult to provide an accurate estimate of the total number of occurrences, snake chomp continues to be a prevalent medical problem in a number of different countries. It is estimated that the number of people who actually suffer from snake envenomation each year could be higher than 5 million. Approximately one million of these are responsible for the intense scream. The variations in the epidemiological data that can be found all over the world are a reflection of the variations in health and exactness that can be found everywhere, as well as the variations in economic and natural conditions. In most situations, access to precise records is restricted, making it difficult to conduct research on topics such as the transmission of diseases or even the fatality rate of snake bite victims. The dependence on traditional healers and specialists in black magic, particularly in agricultural areas, causes the records kept by clinics to be off by a significant margin in comparison to the actual number. It has been estimated that up to eighty percent of persons who have been nibbled by snakes consult traditional specialists before going to a therapeutic centre. This is the case in the majority of non-industrialized societies. As a result of the delay, there are a few casualties that end up passing away while they are being transported to the hospital [1-3]. Because of the high cost of treatment in emergency clinics and the limited availability of venom serums, residents of rural areas frequently find it more beneficial to seek the advice of local doctors who are renowned for successfully restoring patients who have been bitten by snakes. This evidence demonstrates that the plant-based treatments utilised by the regional specialists are effective, and there appears to be a high rate of resiliency among snakebite patients who have progressed to more advanced clinical phases of venom harmfulness. The purpose of this study is to investigate the anti-venom actions of local plants that are indigenous to the western ghats of India. These plants are found in India. It was discovered that traditional healers in the Indian state of Maharashtra made use of these plants [4-5].

## 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
*Helicteres isora* and *Bixa orellana* were selected for use as anti-venom activities in the present investigation based on an increased writing overview. The written survey provided evidence that the plants selected have the

potential to act as anti-venom agents. Dr. T. Chakraborty, Scientist D and Joint Director of the Botanical Survey of India in Koregaon Park in Pune, India, recognised and authenticated the *Helicteres isora* Grah. and *Bixa orellana* specimens. Both of the voucher specimens were brought to the respective Institutes so they could be stored there. The voucher specimen sample number for the *Helicteres isora* Grah. species is ERINTA-2, and the voucher specimen sample number for the *Bixa orellana* species is HPUNCT-1. It was possible to acquire the necessary certificates for the authentication. The powder of the *Helicteres isora* and *Bixa orellana* plant was beaten, and the powdered material (250 g) was removed independently using a hot extraction method with petrol ether, chloroform, ethanol (90 percent), and water. The pooled waste products were then centred around a rotating vacuum evaporator immediately after the biomass stores were removed from the system by filtration. The concentrates, with the exception of the water remover, were likewise dried in an oven at a temperature of 80 degrees Celsius. The shower drier was used to dry the water that was removed. After everything was totally dried off, the concentrates were examined [6-7].

### *In vitro* anti-venom Activity

### Phospholipase Activity

The activity of phospholipase A2 was measured using an abnormal hemolytic measure on an agarose-erythrocyte-egg yolk gel plate using the methods described by Gutierrez et al., 1988. Expanding quantities of *Daboia russelli* venom were applied to 3mm wells in agarose gels containing 1.2 percent sheep erythrocytes, 1.2 percent egg yolk as a wellspring of lecithin, and 10mM CaCl2. The venom was measured in micrograms. Slides were incubated at 37 degrees Celsius for a brief period of time, and the widths of the hemolytic coronas were measured. The control wells each had 15 microliters of saline in them. Comparatively, the base aberrant hemolytic part, also known as MIHD, is analogous to a dose of venom, which resulted in the formation of an 11mm-diameter hemolytic halo.[6] By combining a constant amount of venom (g) with a varied amount of plant removes (l), and then incubating the mixture for 30 minutes at 37 degrees Celsius, we were able to determine that the antidote, which is made from plant extracts, is not totally effective in destroying phospholipase. After that, at that time, aliquots of 10 l of combinations were added to wells in agarose-egg-yolk-sheep-erythrocyte gels. Aliquots were applied to the wells. The venom used in control testing does not contain any plant extracts. At 37 degrees Celsius and for twenty hours, plates were baked. It was expressed as the percentage of mg antibodies to mg venom that could reduce the distance across the hemolytic radiance by half in comparison to the impact that was caused by the venom on its own [6, 7].

### Procoagulant Activity

The procoagulant action was investigated using the method described by Theakston and Reid in 1983, with some modifications made by Laing et al. in 1992. Various volumes of venom that had been broken up in 100 l of PBS with a pH of 7.2 were added to human citrated plasma that was heated to 37 degrees Celsius. The coagulation duration was monitored, and the base coagulant dose10 was not fixed in place as the venom component; this resulted in the plasma being thicker within sixty seconds. The control consisted solely of plasma that had been incubated with PBS. For the purposes of maintaining equilibrium, a consistent measure of venom was mixed in with various weakenings of plant isolates. The combinations were incubated for thirty minutes at a temperature of 37 degrees Celsius. After that, at that moment, 0.1 millilitre of the combination was added to 0.3 millilitres of citrated plasma, and the times at which the plasma coagulated were recorded. In the tubes serving as controls, plasma was brooded with either venom or plant extricates on their own. The equilibrium was expressed as effective dose (ED), which is defined as the proportion of l antibody (plant extricates) per mg of venom at which the coagulating time extended by a factor of multiple times in comparison to the thickening season of plasma hatched with two MCD of venom alone [8].

### Fibrinolytic Activity

The plaque test was modified and used instead. The basal fibrinolytic focus was defined as the concentration of venom that prompted a fibrinolytic glow with a width of 10 millimetres. In order to conduct equilibrium testing, a constant amount of venom was combined with varying concentrations of plant extracts and incubated at 37 degrees Celsius for one hour. Following the completion of the hatching process, the mixture was poured into the respective wells of the plaque. Estimates of fibrinolytic coronas were made after 18 hours of incubation at 37 degrees Celsius [9].

## Result and Discussion

### Phospholipase Activity

*Daboia russelli* venom is prepared to deliver hemolytic haloes in agarose-sheep erythrocyte gels during the phospholipase movement, often known as PLA2. About 10 micrograms of Russell's snake venom produced an 11-millimeter-wide hemolytic corona, which is equivalent to one unit (1U/10 micrograms). This demonstrates that the venom of *Daboia russelli* contains the molecules (PLA2) that are capable of lysing sheep Rbcs. Plant extracts were effective at inhibiting the PLA2 subordinate hemolysis of sheep red blood cells, which was triggered by the venom of *Daboia russelli* in a part subordinate manner. We discovered that that 0.14 to 0.18mg for *Helicteres isora* and 0.10 to 0.18mg for *Bixa orellana* plant extracts were able to totally block PLA2 dependent hemolysis of sheep RBC's produced 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) |
|---|---|---|
| PEEHI | 10 (1 Unit) | 0.14 |
| CEHI | | 0.10 |
| EEHI | | 0.10 |
| AEHI | | 0.14 |
| PEEBO | | 0.14 |
| CEBO | | 0.10 |
| EEBO | | 0.12 |
| AEBO | | 0.18 |

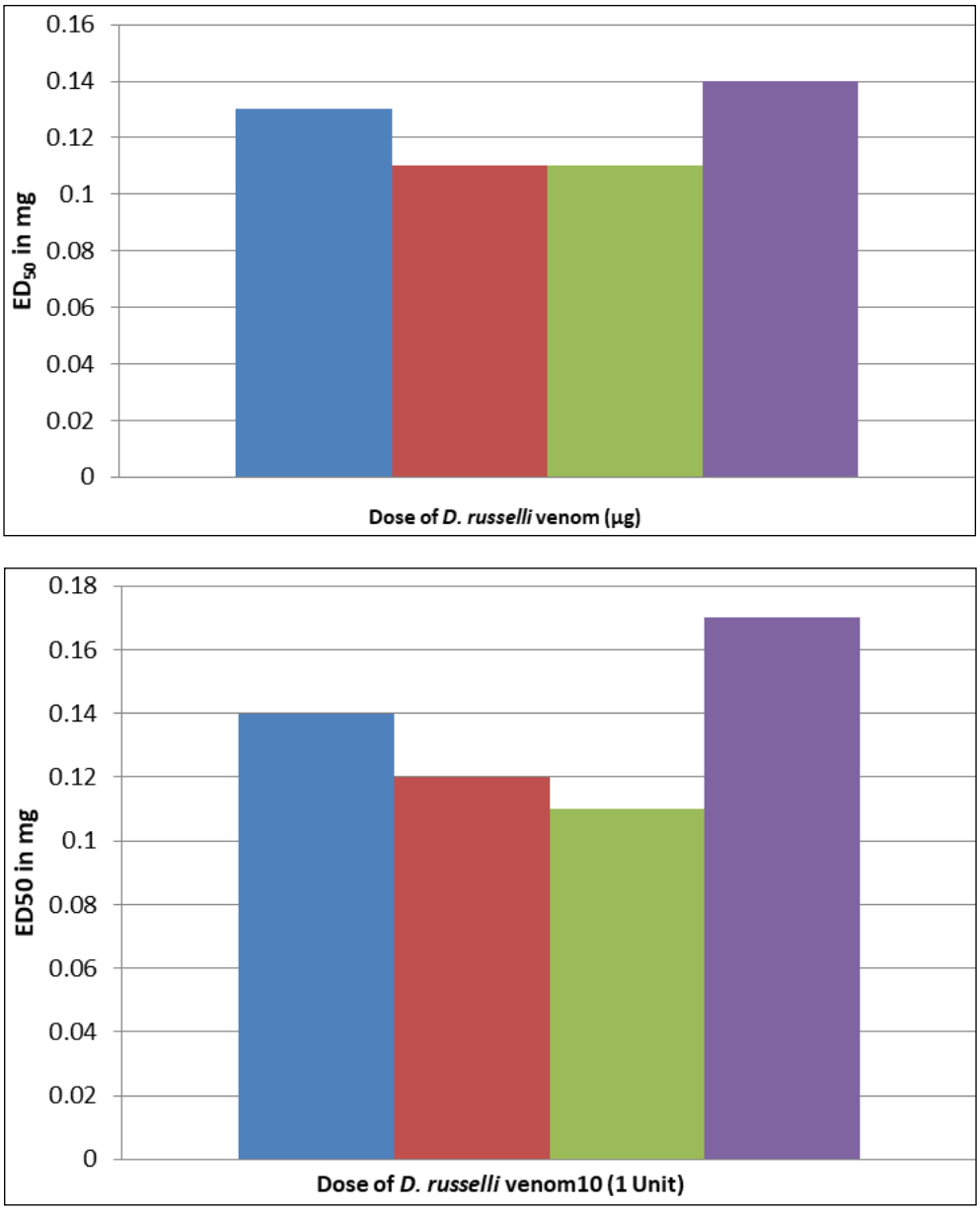


**Fig 1:** Phospholipase activity of *Daboia russelli* venom and its neutralization by plant extracts

**Procoagulant Activity**

The base coagulant element is yet unknown, while the venom component is responsible for the thickening of the plasma in sixty seconds. Within sixty seconds, around 120 grammes of Russell's snake venom thickened human citrated plasma. As part of the equilibrium measurement, the shortage of clump arrangement reveals the respective killing capacities of both plant isolates. According to our findings, 1.6 to 1.9 mg of the plant extract from *Helicteres isora* and 1.4 to 1.9 mg of the plant extract from *Bixa orellana* had the potential to completely stop coagulant movement. The enormous quantity of venom induced rapid thickening, which indicated that a particularly high quantity of antidote was required to be lethal.

**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) |
|---|---|---|
| PEEHI | 16 ($2LD_{50}$) | 1.6 |
| CEHI | | 1.7 |
| EEHI | | 1.8 |
| AEHI | | 1.5 |
| PEEBO | | 1.6 |
| CEBO | | 1.8 |
| EEBO | | 1.4 |
| AEBO | | 1.9 |

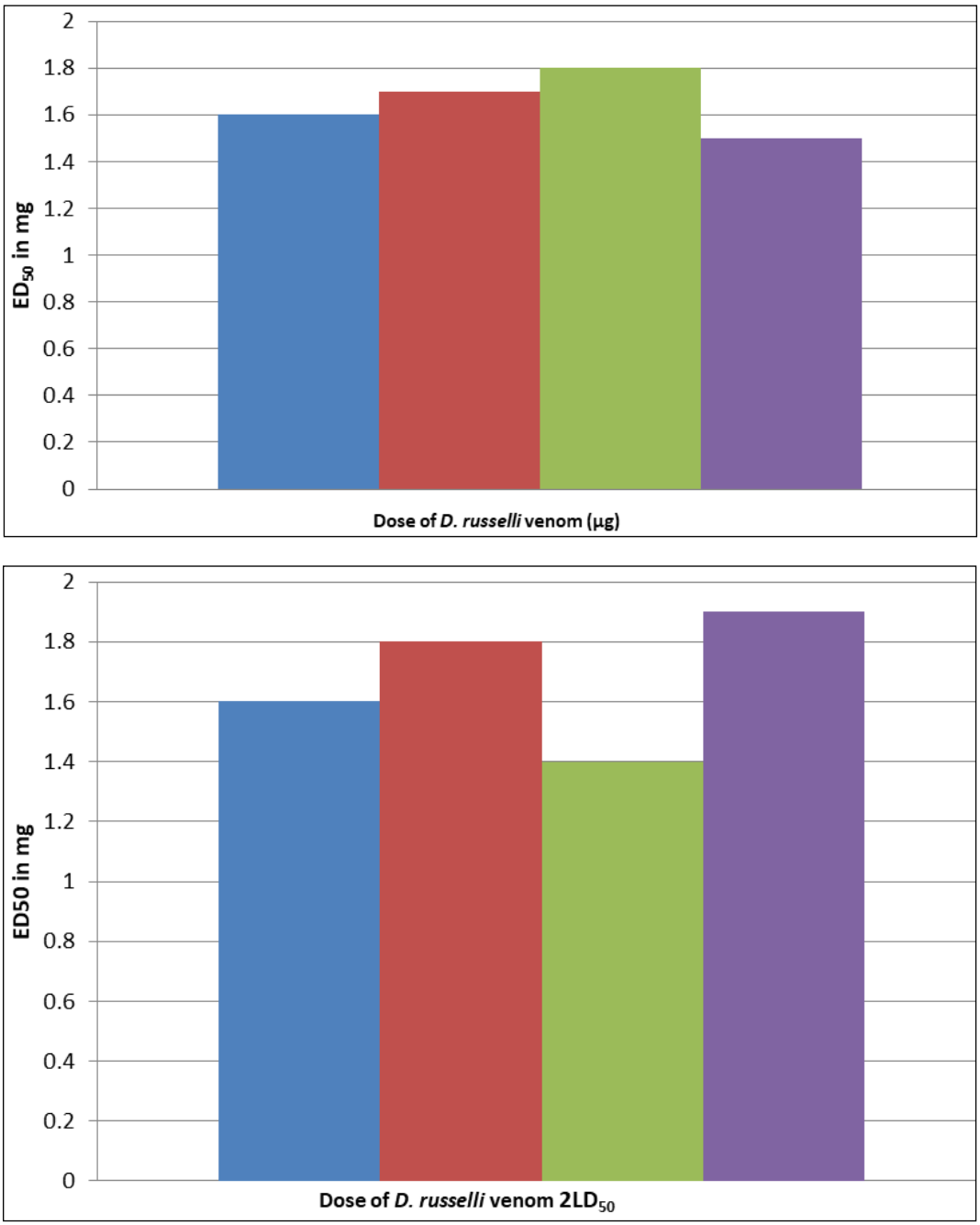


**Fig 2:** Procoagulant activity of *Daboia russelli* venom and its neutralization by plant extracts

**Fibrinolytic Activity**

The plant extracts were effective of suppressing fibrinolytic effect caused by *Daboia russelli* venom. We observed that that 0.10 to 0.18 mg for *Helicteres isora* and 0.14 to 0.18 mg for *Bixa orellana* plant extracts were able to totally suppress fibrinolytic activity (modified plaque assay) generated 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) |
|---|---|---|
| PEEHI | 16 ($2LD_{50}$) | 0.16 |
| CEHI | | 0.10 |
| EEHI | | 0.18 |
| AEHI | | 0.16 |
| PEEBO | | 0.18 |
| CEBO | | 0.18 |
| EEBO | | 0.14 |
| AEBO | | 0.15 |

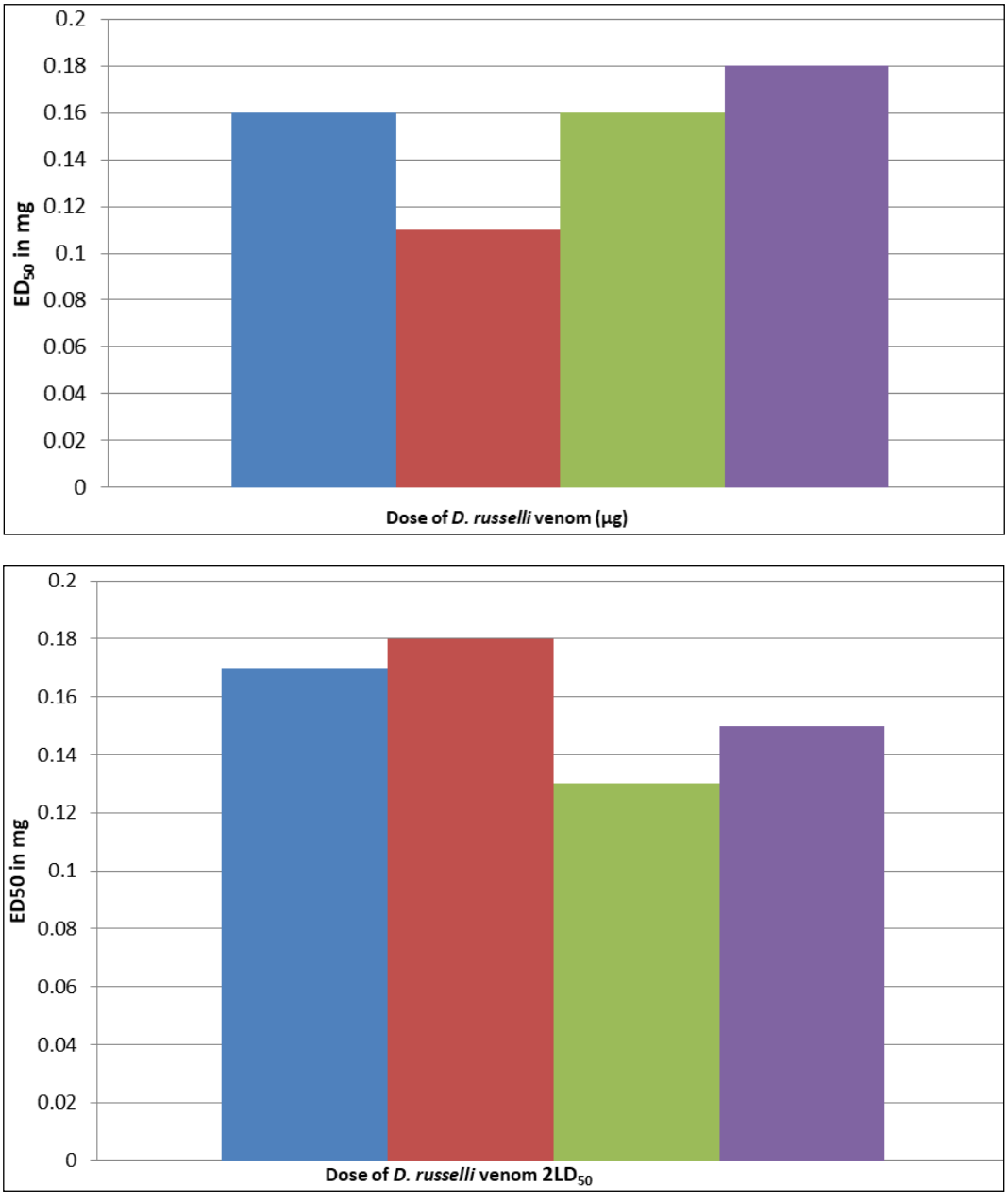


**Fig 3:** Fibrinolytic activity of *Daboia russelli* venom and its neutralization by plant extracts

**Summary and Conclusion**

Plant extracts were capable of inhibiting PLA2 subordinate hemolysis of sheep RBCs, which was caused by the venom of *Daboia russelli* in a part subordinate manner. We believed that concentrations of 0.10 to 0.14 mg of *Helicteres isora* plant extract and 0.14 to 0.18 mg of *Bixa orellana* plant extract had the potential to completely inhibit PLA2 subordinate hemolysis of sheep RBCs caused by *Daboia russelli* venom. The part with the lowest coagulant activity was not completely established as the venom component that caused the coagulation of plasma in the 1960s. According to our findings, a concentration of plant extracts ranging from 1.5 to 1.8 mg for *Helicteres isora* and 1.6 to 1.9 mg for *Bixa orellana* has the potential to completely eliminate coagulant effect. A

significant amount of the venom led to a rapid thickening, which demanded an unusually significant amount of the antidote in order to be fatal. The plant extracts were effective at inhibiting the fibrinolytic response that was brought on by the venom of *Daboia russelli*. We came to the conclusion that concentrations of plant extracts ranging from 0.10 to 0.18 mg for *Helicteres isora* and 0.14 to 0.18 mg for *Bixa orellana* had the potential to completely inhibit fibrinolytic action, also known as an altered plaque measure, brought on by *Daboia russelli* venom.

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