

In-vitro Antitoxin Activity of *Pittosporum neelgherrense* Aqueous Bark Extract against *Daboia russelii* Venom

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Abstract Snake envenomation is a severe and significant global health problem. Until now polyvalent horse antivenom is the only treatment available for snake bite. Many side effects like anaphylaxis, pyrogen reactions and serum sickness is seen on administering horse antivenom. The ancient ayurvedic system of India refers to the usage of plants in management of snake envenomation. In the present investigation, antitoxin activity of *Pittosporum neelgherrense* aqueous bark extract was tested by in-vitro approaches on *Daboia russelii* venom. The bark extracts from *P. neelgherrense* were used to evaluate the in-vitro neutralization tests such as protease, direct haemolysis assay, phospholipase activity, procoagulant, phosphomonoesterase and phosphodiesterase activity. Gas chromatography-mass spectrometry (GC-MS) analysis from bark extracts was also performed to investigate the bioactive compounds. The *in vitro* neutralization assays showed the aqueous bark extract contains some bioactive compounds which effectively neutralises the venom induced activities. GC-MS analysis of the plant extract revealed the presence of antivenom compounds which have neutralizing properties on *D. russelii* venom.

Keywords: *P. neelgherrense*, *D. russelii*, phospholipase, antivenom

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1. Introduction

A major health concern throughout the World especially in India is snake bite due to its high mortality. The incidence of snake envenomation exceeds 5 million per year [1]. *Naja naja*, *Bungarus caeruleus*, *D. russelii* and *Echis carinatus* are the most common poisonous snakes in the country. Among this *D. russelii* and *E. carinatus* belongs to Viperidae and *Naja naja* and *Bungarus caeruleus* belongs to Elapidae [2]. The venoms of *D. russelii* and *Echis carinatus* are histotoxic and hemorrhagic. They cause hemorrhagic and histotoxic manifestations. Currently the only treatment for snake bite is Polyvalent horse antivenom which has adverse side-effects like anaphylactic shock and serum sickness [3]. In remote areas traditional healers treat people with plants that has the metabolites in neutralizing the pharmacological activities of venom [4]. Many Indian medicinal plants are reported for antitoxin activity against snake venoms by different authors [5,6]. *P. neelgherrense* Wight & Arnot, a small evergreen tree is endemic to south western Ghats, India [7]. The bark of this tree is used in folk and tribal medicine as antidote to the snake poison by the Malayar and Malapandaram tribe [8]. Dried stem bark

decotion is administered for poisonous bites, dysentery and chest pain [9]. From the last few decades, phytochemical screening of various *Pittosporum* species has been underway. Several physiologically potent chemical elements such as triterpenoids, flavonoids, glycosides, sesquiterpenes, saponins, carotenoids, and essential oils were discovered as a result of these investigations [10]. Phytochemicals such as saponins, triterpenoids, carotenoids, and essential oils were found in several *Pittosporum* investigations from various locales [11]. The different species of *Pittosporum* (*P. dasycaulon* and *P. floribundum*) were used against inflammation and an anti-dote for snake poison etc. [12]. On the traditional basis if a plant is selected then the herbal drug should be prepared as done by traditional healer [13]. Hence, the present study explores the antitoxin activity of aqueous bark extract of the plant *P. neelgherrense* against snake envenomation.

2. Material & Methods

2.1. Venom

Lyophilized venom of *D. russelii* was obtained from Irula Snake Catchers Industrial Cooperative Society limited,

Vedanemmeli village, Chennai and was stored at 4°C. Stock solution was prepared by dissolving 10 mg of lyophilized venom in 1ml of physiological saline (0.154M NaCl).

2.2. Authentication of Plant

P. neelgherrense (bark) belongs to the family of Pittosporum and was collected from Vanamoolika Herbal Research Foundation, Wayanad, Kerala. It was authenticated by Wayanad Vanamoolika Samrakshana Sangham with the accession No: 182006.

2.3. Preparation of Extract

The bark of the tree *P. neelgherrense* was collected from Vanamoolika Herbal Research Foundation. It was cleaned with running tap water to remove the adhering soil particles. The outer skin of the bark was peeled without using any metal objects and crushed with mortar and pestle and 20 grams was weighed and dissolved in 180 ml of distilled water and incubated overnight in shaker incubator. Next day the extract was filtered using filter paper (Whatman No. A1) and the extracts were evaporated to dryness under reduced pressure below 40°C. The bark extracts were expressed in terms of dry weight.

2.4. Phytochemical Analysis of Extract

Phytochemical analysis of the bark extract was done to check for the presence of various constituents such as alkaloids (Raaman, 2006) [14], flavonoids, saponins (Kokate, 1999) [15], phenols, proteins, coumarins, reducing sugars (Iyengar, 1995) [16], tannins (Mace, 1963) [17], phytosterols (Ismail *et al.*, 2014) [18], triterpenoids (Albri *et al.*, 2014) [19] and anthraquinones (Ayoola *et al.*, 2008) [20].

2.5. Thin Layer Chromatography

The bark extract was concentrated under vacuum and separation of compounds was done by preparative TLC. Concentrated extract was dissolved in methanol and applied on TLC plate with capillary tube with solvent system mobile phase 100 % methanol HPLC grade stationary phase silica gel G coated on TLC plate of 20 X 20 cm. After a run of 15 cm the plates were dried on hot air oven and separated bands were visualized in iodine vapour. Separated bands of compounds in silica is taken into a beaker and 50 ml of pure methanol was added and run in magnetic stirrer for 8 hrs with bead and allowed to stand for 2 hours and the clear filtrate was collected, concentrated and subjected for Gas chromatography-mass spectrometry (GCMS).

2.6. Gas Chromatography-Mass Spectrometry (GCMS)

The GCMS analyses were made in QP-2010 (Shimadzu, Japan). 1 µl of aqueous bark extract of *P. neelgherrense* was injected into GCMS on a 30 m DB-1 capillary column with a film thickness of 0.25 µm (30 m X 0.32 mm i.e. coated with DB-1 using the following temperature programme, initial oven temperature of 70°C for 35 min. The gas chromatography was equipped with Quadrupole

detector. The GCMS was under the computer control at 70 eV. The chromatogram obtained from the GC was then analysed in the MS to get the mass of all the fractions. Interpretation of mass spectrum of GCMS was done using the database of National Institute Standard and Technology (NIST). The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt library.

2.7. Protein and Enzymatic Assays of *D. russelii* Venom

The total protein content present in *D. russelii* venom was estimated by the method described by Lowry *et al.*, 1951 [21] using BSA as a standard. The protein content was read by measuring optical density at 750 nm. The protease enzyme present in *D. russelii* venom was assayed by Yang, 1965 using McIlvaine's citrate buffer and TCA. Briefly, 10 µl of diluted *D. russelii* sample was made up to 0.5 ml with physiological saline and used for the assay. Mixture containing 0.5 ml of McIlvaine's citrate buffer 0.5 ml of *D. russelii* venom sample, 1 ml of 1 % casein was incubated at room temperature for 20 minutes. At the end of the incubation period hydrolysis of casein was stopped by the addition of 4 ml of 5 % TCA to the mixture. The mixture was incubated at room temperature for one hour. At the end the mixture was filtered and the amount of tyrosine liberated was calculated by Lowry *et al.*, method. The OD was read at 750 nm and the protease activity was estimated.

Acetyl cholinesterase enzyme assay was done by the method of Elman (1961) [22]. Briefly, 280 µl of distilled water, 50 µl of 1M KP04 buffer, 100 µl of 75mM ATCI were added to a tube and labelled as test. 50 µl of 1M KP04 buffer, 10 µl of 10 mM DTNB, 50 µl of venom sample, 20 µl of 75 mM Acetyl thiocholine iodide were added to a test tube and served as control. For tube containing all the above reagents except venom, served as blank. The tubes were incubated for 10 minutes. After incubation, 500 µl of 10mM Eserine was added to all the tubes and reaction was stopped. The optical density was measured at 412nm.

2.8. Protein Profile of *D. russelii* Venom by SDS-PAGE

Protein profile of *D. russelii* venom was studied on 10% polyacrylamide gel electrophoresis described by Lamelli (1970) [23]. Venom sample of 15 µl was treated with sample treatment buffer in the ratio of 3:1, heated in the boiling water bath for two – three minutes for the complete interaction between protein and SDS. Samples were cooled and 20 µl of the solution was taken and loaded into the well and the current was turned to 15 mA for 15 minutes and the run was continued at 30 mA until the bromophenol blue reaches the bottom of the gel. The protein profile was visualised by Coomassie blue staining.

2.9. Direct Haemolysis Assay

The haemolytic action of *D. russelii* venom was studied invitro by using RBC. Briefly, 5ml of citrated blood was

centrifuged for 10mts at 900 rpm. The supernatant was poured off and the pellet was washed twice with physiological salt solution. 500µl of physiological saline and 50µl of RBC mixture served as a control. 500µl of distilled water with 50µl of washed RBC was used for 100% haemolysis. 500µl of physiological saline containing 50µl of washed RBC and venom, 50µl of various concentrations of *D. russelii* venom was used to check the venom induced haemolysis. The tubes were put in a thermostat for 1hr at 37°C and centrifuged at 2000 rpm for 20 minutes. The supernatant fluid was poured off to separate tubes to measure the optical density using spectrophotometer at a wave length of 540 nm against water. The calculation of haemolysis was done by the formula.

Experimental sample

$$-(\text{Control sample} \times 100) / 100\% \text{ haemolysis}$$

In neutralization studies 500µl of physiological saline mixed with 50 µl of RBC, 50 µl of venom, with 50 µg, 100 µg, 150 µg, and 200 µg of bark extract was put in a thermostat for 1hr at 37°C and centrifuged at 2000 rpm for 20 minutes. The supernatant fluid was poured off to separate tubes to measure the optical density using spectrophotometer at a wave length of 540 nm against water. The calculation was done by using the above formula.

2.10. Indirect Haemolysis Assay (PLA2 Activity)

Phospholipase activity was measured using an indirect haemolytic assay (Gutierrez *et al.*, 1998) [24]. Increasing concentrations of *D. russelii* venom in 10 µl of saline added to 3 mm wells in Agarose gels (0.8 % in PBS, pH 8.1) containing 1.2% sheep erythrocytes, 1.2 % egg yolk as a source of lecithin and 10 M CaCl₂. Slides were incubated at 37°C and the diameters of haemolytic halo was measured. 10 µl of saline served as control. One unit corresponds to a concentration of venom which produced a haemolytic halo of 11 mm diameter.

In neutralization studies, the mixtures of constant amount of venom were incubated with 20 µg, 40 µg, 60 µg and 80 µg of bark extract, in microliters, for 30 minutes at 37°C and 10 µl of each venom mixture was added to the wells in agarose-erythrocyte-egg yolk gels.

2.11. Coagulant Activity

The procedure described by Theakston and Reid (1983) [25] & Gene *et al.*, was followed to determine the coagulant activity. Various amounts of *D. russelii* venom dissolved in 0.1 ml of human citrated plasma at 37°C. Coagulation time was recorded and the minimum coagulant dose (MCD) was determined as the venom concentration inducing clotting of plasma in 60 seconds. Plasma incubated with PBS served as controls.

In neutralization studies One MCD of venom was incubated with 50 µg, 100 µg, 150 µg and 200 µg, of bark extract in micro liters for 30 minutes at 37°C and 10 µl of each venom mixture was added to 0.1ml of citrated plasma and their clotting times were recorded.

2.12. Proteolytic Activity

Skim milk agar plates (1%) was prepared. Agar wells (2 wells per plate) were cut and 20 µl of *D. russelii* venom was added to the plate and incubated for 24h at 37°C. 20 µl of PBS alone served as a control. Zone of hydrolysis of casein on milk agar plate was measured. For neutralization assay 200 µg, 250 µg, and 300 µg of bark extract were pre-incubated with same concentration of venom for 1 hour at 37°C and these pre incubated samples were added to skim milk agar plates and incubated for 24h at 37°C. Zone of hydrolysis of casein on milk agar plates was measured. Neutralization expressed as the ratio, mg bark extract/mg venom able to reduce by 50% the diameter of the zone of hydrolysis when compared to the effect induced by venom alone was measured.

2.13. Phosphomonoesterase Activity

The procedure described by Bessy *et al.* (1946) [26] with some changes was followed to determine the phosphomonoesterase activity. To 0.25% of venom, 1.0 ml of Tris- Hcl buffer (pH 8.0), 1 ml of disodium -p-nitro phenol phosphate was added and incubated at 37°C for three hours. The absorbance was measured at 425 nm. One unit of enzyme activity was defined as the amount that yielded 0.1 µ mole of p-nitro phenol/hour under experimental conditions. Venom was pre-incubated with 200 µg, 250 µg, and 300 µg of extracts for 30 minutes at 37°C for inhibition studies.

2.14. Phosphodiesterase Activity

The method described by Lo *et al.* (1966) was followed to determine the phosphodiesterase activity. To 0.1 ml of venom, 0.5 ml of 0.0025 M Na- p nitro phenol phosphate, 0.3 ml of 0.01 M MgSO₄ and 0.5 ml of 0.17 M Tris- Hcl (pH 8.0) was added and the absorbance was measured at 400 nm. Phosphodiesterase activity was expressed in Nano moles of product released/minute. Molar extinction coefficient at 400 nm was 8100 Cm-1M-1. The venom was preincubated with 200 µg, 250 µg, and 300 µg of extracts for 30 minutes at 37°C.

3. Results

3.1. Protein and Enzymatic Assays of *D. russelii* Venom

The total protein content of *D. russelii* venom was found to be 80 %. In protease activity 15.2 µg of tyrosine was liberated /ml/min. and acetyl choline esterase activity was found to be nil (Table 1).

Table 1. Protein and Enzymatic assays of *D. russelii* venom

S.NO	NAME OF THE TEST	RESULT
1	Total Protein	80 %
2	Protease Activity	15.2 µg of tyrosine liberated /ml/min
3	Acetyl choline esterase activity	Nil

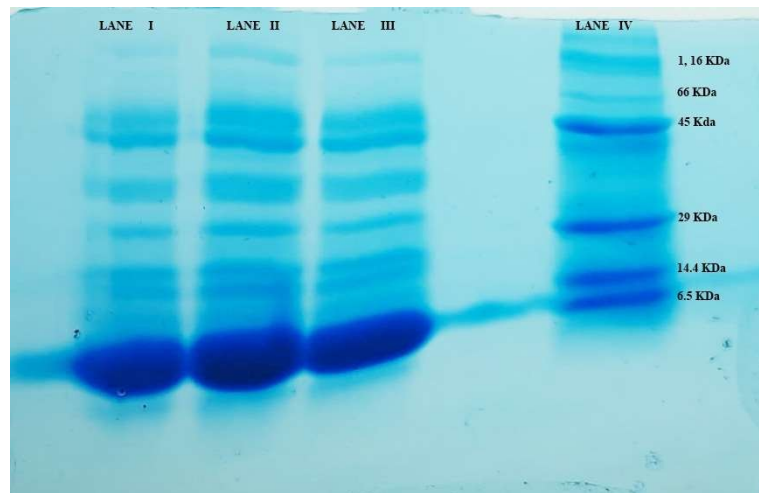


Plate 1. SDS PAGE showing different protein bands of *D. russelii* venom

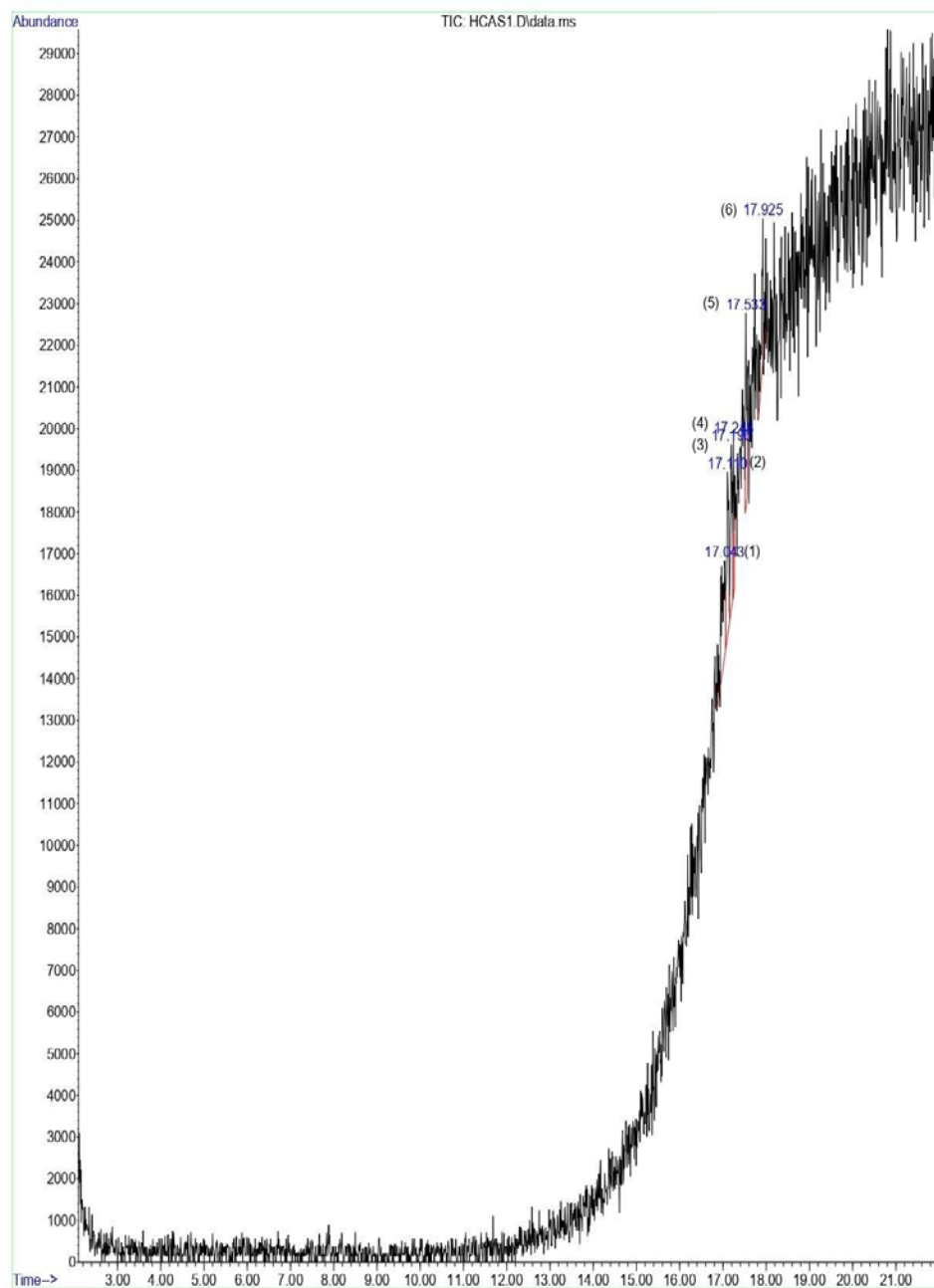


Figure 1. GCMS analysis of aqueous bark extract of *P. neelgherrense*

3.2. Protein Profile of *D. russelii* Venom

The protein profile of *D. russelii* venom was visualised by Coomassie blue staining. In lane I, II and III venom samples were added and in lane IV Marker was added. Different protein bands were observed in SDS – PAGE (Plate 1).

3.3. Phytochemical Analysis of Extract

Phytochemical study from the bark extract revealed the presence of various components. Alkaloids, saponins, protein, tannins, triterpenoids and anthraquinones was found to be present.

3.4. Thin Layer Chromatography

The bark extracts were subjected to TLC. Chromatograms showed significant presence of several types of tannins, flavonoids, coumarins, quinones, carotenoids, saponins, alkaloids and terpenes in *P. neelgherrense*.

3.5. Gas Chromatography-Mass Spectrometry (GCMS)

The results pertaining to GCMS analysis of the aqueous extract of *P. neelgherrense* lead to the identification of different compounds by comparing their retention values with those of known compounds together with mass spectrometry in a NIST library via spectrum matching. The various components present in the bark extract of *P. neelgherrense* that were detected by the GCMS (Table 2).

3.6. In-vitro Neutralization Assays

3.6.1. Direct and Indirect Haemolysis Assay

Direct haemolysis of *D. russelii* venom produced 91.66% hemolysis. Aqueous bark extract neutralized the hemolysis of RBCs produced by the venom up to 21.87%. (Table 3a and Table 3b). In indirect haemolysis assay (phospholipase activity) 20 µg of *D. russelii* venom produced haemolytic halo of 20 mm diameter. 80µg of *P. neelgherrense* bark extract are capable of neutralising *Daboia* venom induced haemolysis (Plate 2).

Table 2. List of compounds present in *P. neelgherrense* aqueous bark extract by GCMS

Peak No.	Retention Time (Min)	Name of the compound
1.	17.045	Silicic acid
2.	17.111	N-Methyl-1-adamantaneacetamide
3.	17.196	1,1,1,3,5,5,5-Heptamethyltrisiloxane
4.	17.244	trimethyl[5-methyl-2-(1-methylethyl) phenoxy] Silane
5.	17.537	2'-(trimethylsiloxy)Propiophenone
6.	17.924	Hexamethyl Cyclotrisiloxane

Table 3a. Direct Hemolysis Activity of *D. russelii* venom

S.NO	Sample	OD of hemolysis	OD of control (RBC +PBS)*	OD of Distilled water % of (100% Hemolysis)	
1	50 µl (50 µg) of <i>D. russelii</i> venom	1.04	0.06	1.08	90.74
2		1.03	0.03	1.08	92.59
3		1.02	0.03	1.08	91.67

Table 3b. Direct hemolysis Activity – Neutralization assay of bark extract of *P. neelgherrense*

S.NO	Venom +Bark extract (150 µg)	OD of hemolysis	OD of control (RBC +PBS)	OD of Distilled water % of (100% Hemolysis)	% of hemolysis
1	<i>D. russelii</i> + Bark extract	0.40	0.04	1.60	22.50
2		0.42	0.07	1.60	21.88
3		0.39	0.05	1.60	21.25



Plate 2. Phospholipase activity (A - Bark extract; B - Venom sample)

3.6.2. Coagulant Activity

In coagulant activity 120 µg of *D. russelii* venom was able to clot human citrated plasma in 60 seconds. 150 µg of aqueous bark effectively neutralised the venom induced coagulant activity.

3.6.3. Proteolytic Activity

In proteolytic activity 20 μg of *D. russelii* venom produced zone of clearance of 10 mm diameter. *P. neelgherrense* bark extract effectively neutralized the venom induced zone of clearance.

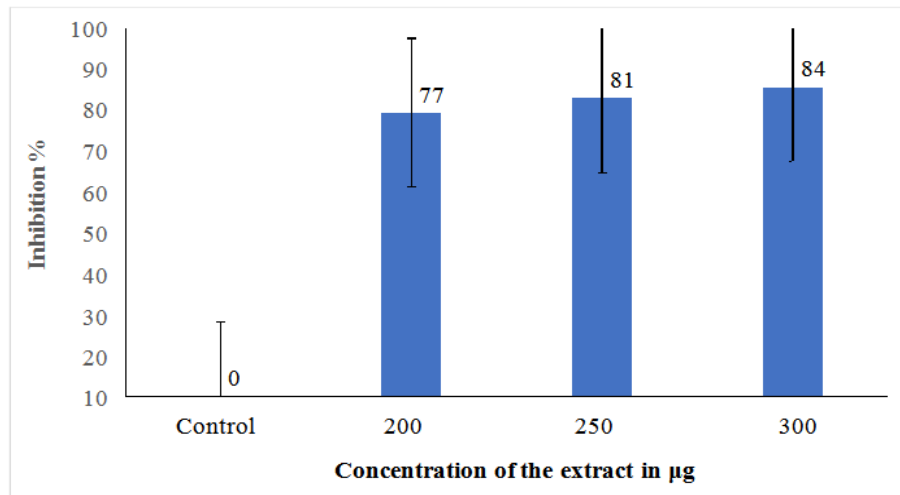


Figure 2. In vitro Neutralization Assay of Proteolytic activity by bark extract of *P. neelgherrense*

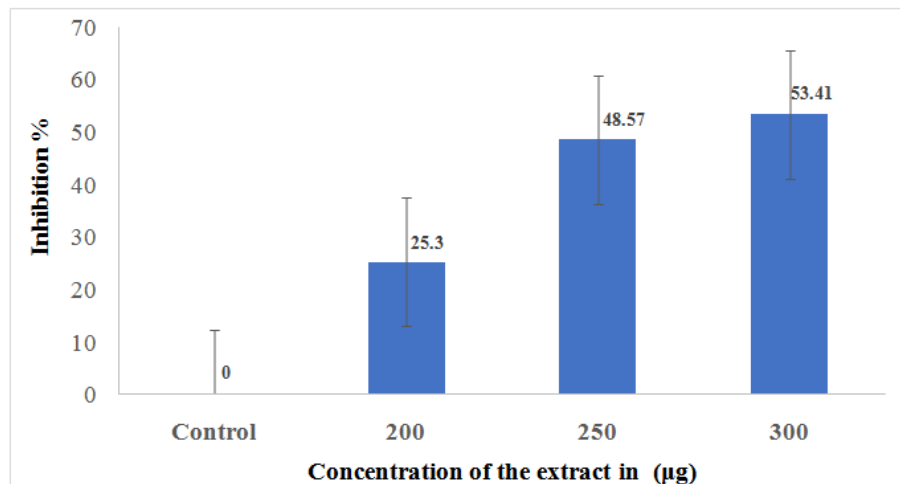


Figure 3. In vitro neutralization Assay of Phosphomonoesterase activity by bark extract of *P. neelgherrense*

3.6.4. Phosphomonoesterase Activity

Enzymatic and inhibition studies revealed that the aqueous bark extract of *P. neelgherrense* was able to inhibit phosphomonoesterase activity.

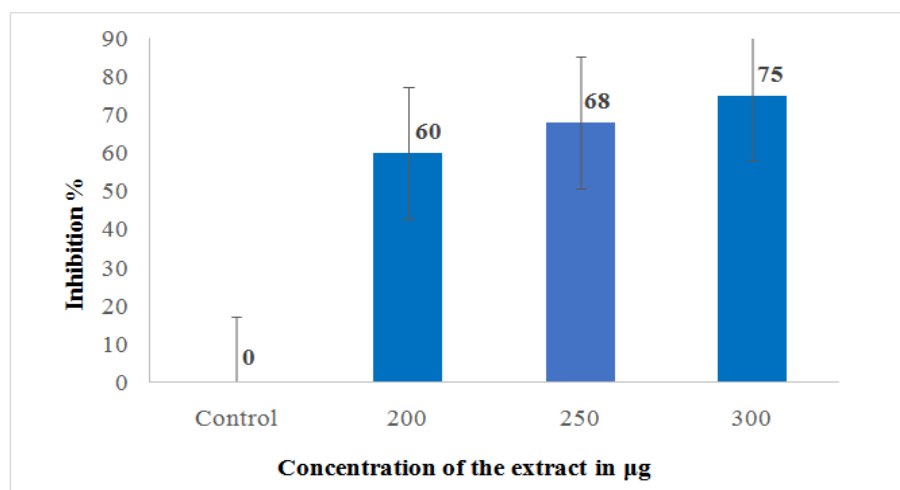


Figure 4. In-vitro Neutralization Assay of Phosphodiesterase Activity by bark extract of *P. neelgherrense*

4. Discussion

Snake envenomation is a significant health threat that prompts high death rate especially in India. It causes pathophysiological symptom which includes damage of the local tissue, edema, diarrhea, vomiting etc. *Daboia russelli* and *Naja kouthia* are the normal snakes found in and around India and a vast number of deaths happen because of envenomation by these snakes [27]. Antivenom remains the main stay for the treatment of snake bite and it is derived from horse sera but it is very less effective and require high dosage [28]. In the present study, *Pittosporum neelgherrense* (bark) is used for neutralizing the *Daboia russelli* venom. In the phytochemical study, Phytoconstituents of various types like flavonoids, phenols, alkaloids, lignin, anthraquinones, steroids, tannins, saponins, fixed oils and glycosides were reported from *Pittosporum* [29,30]. These are responsible for many biological or pharmacological activities [31]. Among the various components identified from GCMS, Silicic acid and Hexamethyl cyclotrisiloxane is found to be effective against snake venom. Similar type of work was carried out using *Dillenia Scabrella* by Momin *et al.* (2020) and reported the presence of Silicic acid and Hexamethyl Cyclotrisiloxane by GCMS [32]. Sreedevi *et al.*, (2017) also reported that *Stereospermum tetragonum*, plant extract was able to neutralize the venom induced haemolysis up to 24 % [33]. Chaterjee *et al.*, (2006) also reported that Lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* could significantly neutralize lethality, haemorrhage, defibrinogenation, edema, PLA2 activity induced by *D. russellii* venom [34]. Batina *et al.*, (2000) suggested that myotoxic phospholipases A2 (PLA2) activities was inhibited by the phytochemical 12-methoxy-4-methylvoachalotine isolated from *Tabernaemontana catharinensis* [35]. Alam *et al.*, 2014 reported that *Curcuma aromatica*, *Aristolochia indica*, *Andrographis paniculata* and *Curcuma zeodaria* antagonised the venom induced plasma recalcification [36]. Ghag-Sawant *et al.*, 2016 reported that the methanolic extracts of seeds of *Pongamia pinnata*, fruits of *Piper longum* and *Sapindus laurifolius* and leaves of *Adhatoda zeylanica* effectively antagonised the proteolytic activity of *D. russellii* venom [37]. Janardhan *et al.*, 2014 suggested that ethyl acetate extract of the plant was able to inhibit phosphodiesterase activity against *Bungarus caeruleus* and *D.russellii* venom [38]. Sani *et al.*, (2020) demonstrated that the hexane fraction of *Azadirachta indica* leaf extracts significantly inhibited the phosphodiesterase activity [39]. From the various results of invitro tests, it was concluded that the bark extract can be used as antivenom.

5. Conclusion

The present investigation shows the ability of aqueous bark extracts to neutralize biological effects of *D russellii* venom. The invitro analysis like phospholipase activity, direct hemolysis activity and procoagulant activity showed that *P. neelgherrense* aqueous bark extract effectively neutralized various toxins of *D russellii* venom. These results suggest that *P. neelgherrense* aqueous bark extract may be used for treatment against *D. russellii* venom.

Further study is needed to explore the exact potential compound responsible for neutralizing the venom.

Conflict of Interest

The authors of this research study declare that they have no conflict of interest.

References

- [1] Chippaux JP (1998). Snake-bites: appraisal of the global situation. Bull World Health Organ. 76(5): 515-524.
- [2] Meenatchisundaram S, Michael A (2009). Snake bite and therapeutic measures: Indian scenario. Indian J Sci Technol. 2(10): 69-73.
- [3] Biranchi Narayan Mohapatra, Mohanty CBK (2010). Guidelines for Anti-Snake Venom Therapy: Medicine update. 20 p.
- [4] Gupta YK, Peshin SS (2012). Do herbal medicines have potential for managing snake bite envenomation? Toxicol Int.19: 89-99.
- [5] Meenatchisundaram S, Priya grace S, Vijayaraghavan R, Velmurugan R, Parameswari G, Michael A (2009). Antitoxin activity of Mimosa pudica root extracts against Naja naja and Bungarus caeruleus venoms. Bangladesh J Pharmacol. 4:105-9.
- [6] Meenatchisundaram S, Michael A (2010). Antitoxin activity of Mucuna pruriens aqueous extracts against Cobra and Krait venom by in vivo and in vitro methods. Int J Pharm Tech Res. 2: 870-4.
- [7] Mohanan N, Sivadasan M (2020). Flora of Agasthyamala: Bishen Singh Mahendra Pal Singh. New Delhi. India.76-77.
- [8] Binu S, Nayar TS (2005) Pittosporum neelgherrense (Wight & Arnott) [Pittosporaceae] in treatment of snake bite. Economic Botany. 2005, 59: 295.
- [9] Nair GM, Rajasekharan S, George V. (2004). Final Scientific and Technical Report on Pilot Participatory Programme on Conservation and Sustainable Use of Medicinal and Aromatic Plants. Thiruvananthapuram: India: TBGRI Publication. 20 p.
- [10] Semwal P, Anthwal P, Kapoor T, Thapliyal A. (2014). Preliminary investigation of phytochemicals of Saussurea obvallata (brahmkamal) and Pittosporum eriocarpum (agni): two endangered medicinal plant species of Uttarakhand. International Journal of Pharmacognosy. 1: 266-269.
- [11] Feng C, Li BG, Gao XP, Qi HY, Zhang GL (2010). A new triterpene and an antiarrhythmic liriendendrin from Pittosporum brevicalyx. Arch Pharm Res. 33: 1927-32.
- [12] Khare CP (2007). Indian medicinal plants: an illustrated dictionary. Springer – Verlag, Berlin, Germany: 496.
- [13] Fabricant DS, Farnsworth NR (2001). The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect. 109: 69-75.
- [14] Raaman N (2006). Phytochemical Techniques. New Publishing Agency; New Delhi: 19: 24: 32-40.
- [15] Kokate CK (1999). Practical Pharmacognosy. Vallabh prakashan publication. 11-116.
- [16] Iyengar MA (1995). Study of crude drugs. Manipal power press, Manipal; India: 8: 2-5.
- [17] Mace ME (1963). Histochemical localization of phenols in healthy and diseased banana roots. Physiol. Plantarum. 16: 915-925.
- [18] Ismail AM, Mohamed EA, Marghany MR, Abdel-Motaal FF, Abdel-Farid IB, El-Sayed MA. (2014). Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of Faidherbia albida legume extracts. J. Saudi Soc. Agric. Sci. 15: 2: 112-117.
- [19] Alabri THA, Musalami AHSA, Hossain MA, Weli AM, Al-Riyami Q. (2014). Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of Datura metel L. J. King Saud. Univ. Sci. 26: 237-43.
- [20] Ayoola GA, Coker HAB, Adesegun SA, Adepojubello 3AA, Obaweya K, Ezennia EC, Atangbala TO. (2008). Phytochemical Screening and Antioxidant activities of some selected Medicinal plants used for Malaria Therapy in South Western Nigeria. Tropical J. Pharmaceutical Research. 7:3:1019-1024.

- [21] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*. 193, 265-275.
- [22] Ellman LG, Diane KCV, Andresjr R, Featherstone MB. (1961). *Biochemical Pharmacology*. 7: 2: 88-90,
- [23] Laemmli UK. (1970). Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature*. 227: 680-685.
- [24] Gutierrez JM, Avila C, Rojas E, Cerdas L (1988). An alternative in vitro method for testing the potency of the polyvalent antivenom produced in Costa Rica. *Toxicon*. 26: 411-413.
- [25] Theakston RDG, Reid HA (1983). Development of simple standard assay procedures for the characterization of snake venoms. *Bulletin of the World Health Organization*. 61, 949-956.
- [26] Bessy OA, Lowry OH, Brock MJ. (1946). A method for the rapid determination of alkaline phosphates with five cubic millimeters of serum. *J Biol Chem*. 164: 321-9.
- [27] Patel M, Mukund H, Swaroop. (2013). Anti-Venom Activity of *Cinnamomum zeylanicum* Extracts Against *Naja kaouthia* snake venom. *International journal of pharmaceutical and chemical sciences*. 2(3): 1302-1310.
- [28] Shashidharamurthy R, Kemparaju K. (2007). Region-specific neutralization of Indian cobra (*Naja naja*) venom by polyclonal antibody raised against the Eastern regional venom: A comparative study of the venoms from three different geographical distributions. *International journal of immune pharmacology*. 7: 61-69.
- [29] Nagamalleswari K, Yasodamma N, Binny AJR. (2013). Phytochemical screening, antibacterial and antifungal studies of *Pittosporum floribundum* Wight & Arnott leaf, bark, fruit and seed extracts. *Journal of pharma & biosciences*. 4: 464-474.
- [30] Mani B, Dennis T. (2014). Evaluation of the antioxidant potential of *Pittosporum dasycaulon* Miq. stem bark. *Food Science and Biotechnology*. 23: 539-545.
- [31] Rungsung W, Ratha KK, Dutta S, Dixit AK, Hazra J. (2015). Secondary metabolites of plants in drugs discovery. *World J Pharm Res*. 2015; 4: 604-13.
- [32] Momin K, Thomas SC. (2021). GC-MS analysis of antioxidant compounds present in different extracts of an endemic plant *Dillenia scabrella* (dilleniaceae) leaves and barks. *International Journal of Pharmaceutical Sciences and Research*. 11(5): 2262-2273.
- [33] Sreedevi NS, Thulasi sivaraman, Meenatchisundaram S, Vadivelan R (2017). Invivo and invitro neutralizing potential of *Stereospermum tetragonum* plant extract against Russell's viper (*Daboia russelii*). *Int. J. Res. Ayurveda Pharm*. 8(2): 266-270.
- [34] Chatterjee I, Chakravarty AK, Gomes A. (2006). *Daboia russelii* and *Naja kaouthia* venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* R.Br. *Journal of Ethnopharmacology*. 106: 38-43.
- [35] Batina M, Cintra FA, Veronese EL, Lavrador MA, Giglio JR, Pereira PS, Dias DA, França SC, Sampaio SV (2000). Inhibition of the lethal and Myotoxic Activities of *Crotalus durissus terrificus* Venom by *Tabernaemontana catharinensis*: Identification of One of the Active Components. *Planta Med*. 66(5), 424-428.
- [36] Alam MI. (2014). Inhibition of toxic effects of viper and cobra venom by Indian medicinal plants. *Pharmacol Pharm*. 5: 828-37.
- [37] Ghag-Sawant MT, More V, Samant LS, Chowdhary AS. (2016). Study of neutralization of enzymatic activity of *Daboia russelii* venom by various plant extracts and their combinations using in vitro methods. *International Journal of Pharmaceutical sciences and research*. 7 (6): 2531-2536.
- [38] Bhavya Janardhan1, Vineetha M, Shrikanth1, Kiran KM, Sunil SM (2014). In vitro screening and evaluation of antivenom phytochemicals from *Azima tetraantha* Lam. leaves against *Bungarus caeruleus* and *Vipera russelli*. *Journal of Venomous Animals and Toxins including Tropical Diseases*. 20: 12.
- [39] Sani I, Rabi AU, Sanusi WH, Umar ZF, Fatima BI, Abubakar A1. (2020). Inhibition of Snake Venom Enzymes and Antivenom Adjuvant Effects of *Azadirachta indica* A. Juss. (Meliaceae) Leaf Extracts. *European Journal of Medicinal Plants*. 31(10): 114-128.

