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Research Article

ANTI-PHOSPHOLIPASE ACTIVITY OF MEDICINAL PLANTS AGAINST *NAJA NAJA* VENOM

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ABSTRACT

The aim of the study is to scientifically validate the anti-phospholipase activity of medicinal plantsAndrographis paniculata and Aerva lanata against Naja naja venom. The in-vitro Phospholipase, lytic and procoagulant assay are carried using Naja naja venom and plant extracts to check anti-phospholipase, anti-lytic and anti-coagulant activities. Compounds present in the extracts are characterized using Gas chromatography and Mass Spectrometry (GC-MS) and the compounds obtained from GC-MS are subjected to docking studies with Phospholipase A2. C-terminal residues (Ile104, Ala101), α- helix 3 (Val 47, Phe 46) and α- helix 4 (Asp122, Pro121) of PLA2 form binding moieties, were revealed by molecular docking analysis of phyto compounds in different plants against PLA2 molecule. The binding affinity of various compounds with Phospholipase A2 receptor showed that the activity of PLA2 in *Naja naja* venom can be blocked. The compounds from these plants can be used for developing novel medicine for snake bite which is of *ethno pharmaceutical origin.*

Keywords: *Andrographis paniculata; Aerva lanata;* TLC; GC-MS; Phospholipase A2.

INTRODUCTION

According to WHO estimation in 2011, five million people suffer from snake bite resulting in 1,00,000 deaths and 3,00,000 cases of permanent impairment in India alone. There are five families of poisonous, which are Colubridae, Elapiadae, Hydrophidae, Viperiade and Ataspididae[1]. Spectacled cobra (*Naja naja*), common krait *(Bungarus caeruleus)*, saw-scaled viper *(Echis carinatus)* and Russell's viper (*Daboia russelii*) are the most important widely spread venomous snakes in India. Most of the potent snake venom is highly specific to their targets, such as the neuromuscular junction or components of the haemostatic system[2].

The *Naja naja* is considered to be medically important snake due to their number of bites and mortality in geographical range. They range throughout Southwest Asia, Central Asia, South Asia, East Asia, and Southeast Asia. *Naja naja* venom consists ofdigestive hydrolases, L-amino acid oxidase, phospholipases, thrombin-like pro-coagulant and kallikrein-like serine proteases and metalloproteinase. The administration of Anti Snake Venom (ASV), the only specific treatment for snake bite, however, is associated with many drawbacks like severe pain, edema, localized hemorrhage and necrosis which often results in permanent scarring and deformity[3].

In recent years, studies on evaluation of the therapeutic and toxic activity of herbal medicinal products became prominent. The better understanding in phyto pharmaceuticals results in the development of rational therapies for snake bite[4]. About 40% of modern monomolecular drugs are derived either directly or indirectly from the plants[5]. Usage of plants as a source for medicine has been inherited from generations. In the Indian system of medicine, most practices include formulation of their own recipes, hence requires proper documentation and research[6]. The ether soluble fraction of *Aristolochia* species inactivates and reduces haemorrhage caused by *Naja naja* venom[7]. Plants like *Schumanniophyton magnificum*,*Eclipta prostrate*, *Caesearia sylvestris* and *Aristolochia shimadai* consists of biochemical compounds which inhibits phospholipase A_2[8]. The persimmon tannin of *Diospyros kaki* is known to have anti-haemorrhagic effect. The leaves of *Munuca pruriens var. utilis*, *Strophanthus grantus* and *Strophantus hispidus* have the ability to prolong the clotting time against *Echis carinatus* venom[9]. Aqueous extracts of dried roots of *Mimosa pudica* inhibited lethality against *Naja kaouthia* venom. It also contains anti-hyaluronidase activity[10]. *Pentaclethra macroloba* exhibited inhibition against edema, myotoxic, PLA_2 and metelloprotease. The whole seed extract of *Strychnus nux vomica* inhibited viper venom induced lipid peroxidation in experimental animals[11]. *Achyranthes aspera* is used in treating snakebite, asthma, and cough. It is pungent, antiphlegmatic, antiperiodic, diuretic, purgative and laxative, useful in edema, dropsy and piles, boils and eruptions of skin,etc[12]. Crushed plant is boiled in water and is used in pneumonia. Infusion of the root is a mild astringent in bowel complaints.

Andrographis paniculata,a medicinal plant of Acanthaceae family is used for treatment of upper respiratory infection, ulcerative colitis and rheumatic symptoms. They are the potential mosquito repellent. The aerial part of the plant is rich in diterpenes, lactones, and flavonoids. *Aerva lanata* serves the purpose of anthelmintic and medication that soothes inflamed or injured skin. It is also in use for the treatment of lithiasis, cough, sore throat, indigestion, wounds and as a specific for diabetes. The leaves are reportedly hepatoprotective[13].The present investigation explored the Phospholipase activity and lytic activity of *Andrographis paniculata and Aerva lanata*.

MATERIALS AND METHODS

Plant collection

Mature and fresh leaves of *Andrographis paniculata, and Aerva lanata were* collected from the Sathyamangalam rural Area. The leaves were washed thoroughly using distilled water. The plant materials were shade dried and powdered[14].

Venom collection and storage

The *Naja naja* snakes were caught around villages of Sathyamangalam, Tamil Nadu, India. The venom was collected from *Naja naja* and stored at -4°C until use. The crude venom was taken as working standard concentration.

Plant sample extraction and storage

10g of dried leaf powder of all the plant were subjected to extraction using acetone as solvent for about 30 cycles and then concentrated using a rotary evaporator and stored in air tight containers at 4° C[15].

Thin Layer Chromatography (TLC) and GC-MS analysis

After the application of the sample on the adsorbent, the TLC plate was kept in the TLC glass container with acetone solvent. The separations of the colored spots were obtained. The R_f values for different spots for different extracts were determined[16]. The separated bands were scrapped from the TLC plates. The scrapped powder was centrifuged with 0.75ml of methanol. After centrifugation, the supernatant was subjected to GC-MS analysis.

JEOL GCMATE II gas chromatograph, photomultiplier detector, Helium at 1 ml/min, SPB- 5 capillary column using split led injector, was used. The ignition temperature was at 220° C, column temperature was maintained at 50° C for the first 10 mins and then raised to 230° C (10° C/min) followed by 240° C. Jeol JMS-HX 110 mass spectrometer with source at 270° C and 70 eV with Quadrapole double temperture mass analyser. The analysis was performed on the aforementioned programme on equivalent column HP-5 (25 m x 0.22 mm and 0.25 µmdf). A mass spectral survey was performed using the NIST mass spectral search program 1998.

Docking

Retrieval of the protein

The structures of target proteins were retrieved from the protein data bank. The PDB ID of the target proteins phospholipase A2 from *Daboia russelii* was 2PYC.

Retrieval of the ligand structures

The phytocompounds obtained from GCMS analysis were used for docking. The 3D structure of these phytocompounds was retrieved from the Chemspider database in the SDF file format which is converted into PDB file format through Open Babel.

Docking analysis

Computer simulated automated docking studies were performed using AutoDock 4.1. It was designed and implemented by Dr. Oleg Trott in the Molecular Graphics Lab at The Scripps Research Institute (La Jolla, CA, USA). Auto Dock tools were used to add polar hydrogen molecule to the receptor. The grid box was set to include the whole receptor region. The receptor output and ligand were saved in PDBQT format, which can be read by using Vina. The binding energy and inhibitory constants were observed and analysed[17].The interactive residues were found using Auto Dock and the closest distance between the atoms were measured in Van der Waals (VDW) scaling factor. The VDW scale for the receptor and the ligand was set to unity.

Phospholipase activity

Phospholipase activity of *Naja naja* venom was measured using Agarose-erythrocyte-egg yolk gel plate method[18]. Fresh human blood was taken and centrifuged at 10,000rpm for 8 min. The supernatant was removed and the pellet was washed four times with PBS solution. A mixture consisting 300µl of human erythrocyte, 300µl of 1.2% egg yolk in saline solution and 250µl of 0.01M $Cacl_2$ solution was added to 25ml of 1% agar solution at 50°C dissolved in PBS .The content was added to the petri plate and allowed to solidify. Later well of about 3mm was made. The various concentration of *Naja naja* crude venom was added to the well and incubated at 37 °C for 20 hour. The Minimum Indirect Haemolytic Dose (MIHD) was defined as the amount of venom that induced a haemolytic halo of 11-mm diameter. The various concentration of the plant extract was added with the MIHD concentration. The control was the venom with PBS solution. Later the diameter of the halos was measured. Neutralization was expressed as the ratio of mg antibodies/mg venom which could reduce the diameter of the haemolytic halo by 50% when compared to the effect induced by venom alone[19].

Procoagulant assay

The Minimum Coagulant Dose (MCD) was determined as the venom dose, which induced clotting of plasma within 60 seconds. The various concentration of *Naja naja* venom was dissolved in100µl PBS solution. To 0.1ml of the diluted venom solution added 0.3ml of citrated plasma. Plasma incubated with PBS alone served as control. The mixture was incubated for 30min at 37 °C. The clotting time was recorded and MCD value was found. Later plant extracts are added at different concentrations to the MCD diluted venom concentration and phosphate buffer. Neutralization was expressed as effective dose (ED), defined as the ratio µl antivenom (plant extracts)/mg venom at which the clotting time increased three times when compared with clotting[20].

Lytic activity

The fresh human blood was collected and centrifuged at 10,000 rpm for 10min. 1% Human Red Blood Cells (HRBC) solution was prepared by washing the pellet three times with PBS solution. Then stock solution of 100µg/ml was prepared by dissolving lyophilized *Naja naja* venom in saline solution. To different concentrations of the plant extracts (10, 20, 30 ,40, 50, 60, 80, 100, 150µg/ml) was added 1ml of 1% HRBC, 1ml of phosphate buffer and 1ml of stock solution. The control comprises mixture without plant extract. The mixtures were incubated for 30min at 37 °C. This was centrifuged at 1000rpm for 3min and theabsorbance value for the supernatant was measured at 540nm. The control gives 100% lysis value. And the inhibition percentage of haemolysis was calculated accordingly[21].

Antiphospholipase activity

The acetone extract of *Andrographis paniculata* and *Aerva lanata* were subjected to Phospholipase activity. Minimum Indirect Haemolytic Dose (MIHD) was stated as the amount of venom that induced a haemolytic halo of about 11-mm diameter. Various concentrations of *Naja naja* venom induced different diameters of haemolytic haloes as shown in figure 1. The MIHD value of *Naja naja* venom was found to be 4µl. Different concentration of

plant extracts against MIHD value (4µl) induced haemolytic halos of various diameters as shown in figure 2. The inhibition of phospholipase can be calculated by 50% reduction of haemolytic halo and was found to be 20µl of *Andrographis paniculata* (5.4mm) and 10 µl of *Aerva lanata* (5.5mm).The acetone extract of *Aerva lanata* was observed to be effective. Thus, haemolytic halo in agarose erythrocyte egg yolk gel plate by *Andrographis paniculata* and *Aerva lanata* proved anti- Phospholipase activity against *Naja naja* venom. The determination of MIHD value in agarose erythrocyte egg yolk gel plate against different concentrations of *Naja naja* venom was mentioned in table 1.

Procoagulant activity

The plant extracts were subjected to procoagulant activity against *Naja naja* venom. Minimum Coagulant Dose (MCD) was determined by the minimum dose required to clot plasma within 60 seconds. The MCD value was observed to be 2µl of *Naja naja* against 300 µl of human citrated plasma as shown in table 2. The leaf extracts showed effective neutralization of coagulant activity of the venom by inhibiting clot formation. Figure 3 shows 40µl of *Andrographis paniculata* (243±1.2 seconds) and 30µl of *Aerva lanata* (238±0.7 seconds) leaf extracts was able to inhibit four times MCD coagulation. Thus from the procoagulant study, *Aerva lanata* was comparatively more effective than *Andrographis paniculata* plants extract.

Anti- haemolysis activity

The neutralization of lytic activity of *Naja naja* venom by *A.paniculata* and *A.lanata* was calculated using OD value at 540nm. The plant extracts were able to inhibit by 50 percentage lytis 40 µl of A.paniculata and 30 µl of A.lanata leaf extracts as shown in figure 4. *A.lanata* showed better anti haemolytic activity when compared to *A.paniculata*.

Thin layer chromatography

The result from the above *invitro* studies confirmed the anti-Phospholipase, anti-coagulant and anti-lysis activity. Further studies were done to determine the compounds responsible for these activities in the plant extract. The acetone extract of *Andrographis paniculata* and *Aerva lanata* were subjected to Thin Layer Chromatography.

Gas Chromatography-Mass Spectrometry analysis

The Separated band 1and 2 as shown in figure 5 were scrapped off and dissolved in methanol for GC-MS analysis. The chromatogram showed8 peaks in *Andrographis paniculata* and 7 peaks in *Aerva lanata* as shown in figure 6 and 7. The extracts consisted of 7 esters, 9 alkanes and 1 organic acid as listed in table 4 and 3.

Auto Docking

The crystal structure of PLA2 Receptor (2PYC) retrieved from the protein data bank and compounds from GC-MS analysis was subjected to docking studies using MGL tools AutoDock, Vina and Pymol. Ester compounds from the acetone plant extract were observed to have minimum binding affinity. The binding affinity of the compounds with PLA2 and the interacting residues were listed in table 3 and 4. Corynan-17.ol.18.19.didehydro-10-methoxy-Acetate in*Andrographis paniculata* andHexadecanoic acid, 1-(hydroxymethyl)- 1,2 ethanediyl ester in *Aerva lanata* as shown in figure 8 and 9 were having minimum binding affinity.

The binding pocket of compounds from acetone extract of *Andrographis paniculata* with the receptor PLA2 (PDB id: 2PYC) lies likely in C-terminal and 3, 4 α- helix and in *Aerva lanata* at C-terminal and α- helix 4. On analyzing the binding pocket of *Andrographis paniculata* and *Aerva lanata* plant extracts with the receptor, the binding pocket of PLA2 was C-terminal end (Ile104, Ala101), α- helix 3(Val 47, Phe 46) and α-helix 4(Asp122, Pro121) as shown in the figure 10. Hence these site forms the binding pocket for compounds from the plants. Mutational studies and protein binding studies with the compounds will give an insight view on the binding affinity.

Table 1: **Determination of MIHD value in agarose erythrocyte egg yolk gel plate against different concentrations of *Naja naja* venom.**

Sl. No.	Concentration of Naja naja Venom (µl)	Zone formation(mm) (n = 3)
1	Control	0
2	2	6.8±0.1
3	3	7.2±0.2
4	4	11.1±0.3
5	5	12.8±0.23
6	6	13.6±0.35

Table 2: **Determination of MCD value of *Naja naja* venom using human citrated plasma.**

Sl. No.	Concentration of *Naja naja* Venom (µl)	Volume of human citrated plasma (ml)	Coagulation time (sec) *(n = 3)*
1	Control	300	0
2	1	300	67±2.0
3	2	300	59±1.2
4	3	300	36.2±1.0
5	4	300	24±0.7
6	5	300	13±1.2

Table 3: Overall binding affinity of compounds present in *Andrographis paniculata*

Ligand	Retention time (min)	Binding affinity	Interacting residues
Pentadecanoic acid, 14-methyl ,- methyl ester	17.27	-2.9	Gln108, Ile104,Ala101
Dasycarpidan- 1 – methanol, acetate (ester)	19.07	-5.3	Leu130, Phe46,Arg43
Oleic Acid	19.77	-3.3	Asp122, Pro121, Phe124
9- Octadecenoic acid (Z); 2-hydroxy – 1 – (hydroxymethyl) ethyl ester	22.07	-4.4	Asp122, Phe124
Hexadecanoic acid, 1-(hydroxymethyl)- 1,2 ethanediyl ester	23.22	-5.3	Trp31, Asp122,Pro121, Ser24, Leu119
9- Octadecenoic acid (Z); 2-hydroxy – 1 – (hydroxymethyl) ethyl ester	26.15	-4.0	Val47, Cys50, Phe46, Arg43, Leu130, Cys133

Table 4: Overall binding affinity of compounds present in *Aerva lanata*

Ligand	Retention time (min)	Binding affinity	Interacting residues
Corynan-17.ol.18.19.didehydro-10-methoxy-. Acetate (ester)	27.98	-5.9	Trp31, Asp122, Phe124, Pro121
Heptadecane.9-hexyl-	28.02	-3.9	Ala101 ,Cys105, Ile104, Val47
1.2-benzenedicarboxylic acid. Bis(2-methylpropyl)ester	15.73	-4.0	Cys105, Ala101
Heneicosane	16.9	-3.7	Trp31, Leu119
Docosane	17.87	-3.9	Asp122,Phe124
Tetracosane	19.7	-3.9	Arg43,Phe46,Val47,Leu130,Cys133
Triacontane	20.75	-3.3	Val47, Ile104,
Tetratetracontane	21.45	-2.7	Leu119,. Pro121,Asp122
Heptacosane	22.03	-3.9	Leu130,Arg43, Phe46, Val47
Octacosane	23.53	-3.5	Lys115,Leu110,Asn111,Arg107
Triacontane	22.03	-3.3	Val47, Ile104

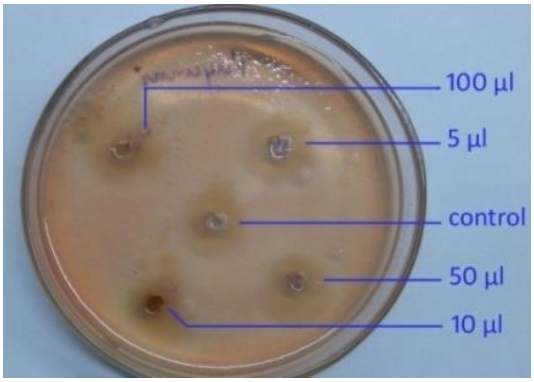

Figure 1: **The anti-Phospholipase activity of Andrographis paniculata against Naja naja venom**.

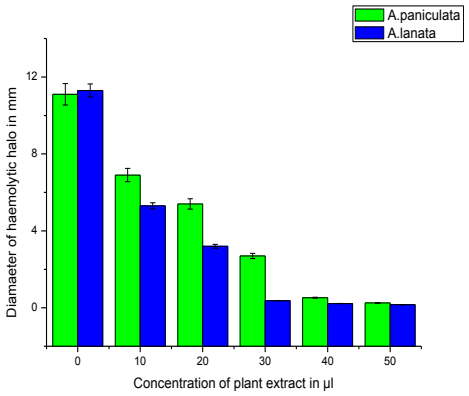

Figure 2: Comparison of Anti-Phospholipase activity of Andrographis paniculata and Aerva lanata against Naja naja venom (n=3).

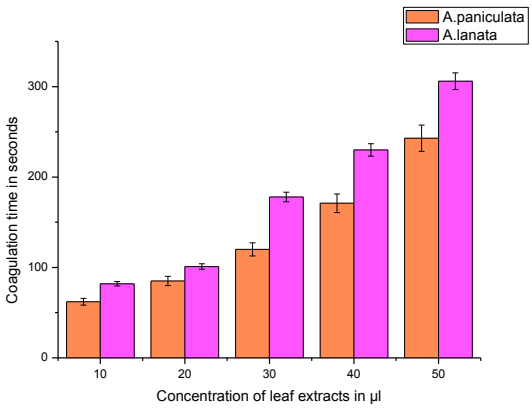

Figure 3: Comparison study of Anti-coagulant activity of Andrographis paniculata and Aerva lanata against MCD (4µl) value of Naja naja venom (n=3)

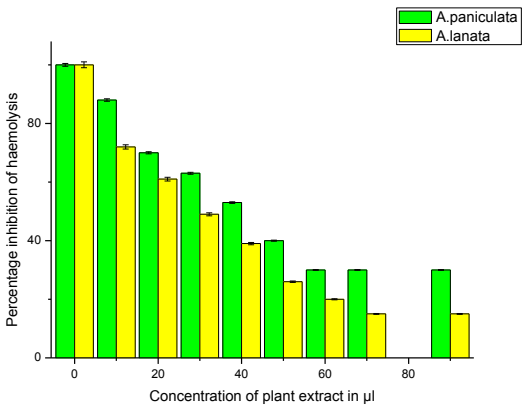

Figure 4: Comparison of anti-lytic activity of Andrographis paniculata and Aerva lanata against Naja naja venom.

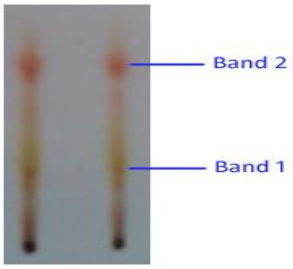

Figure 5: Separate band formation of phyto chemicals in Acetone extract of A.lanata in TLC plate.

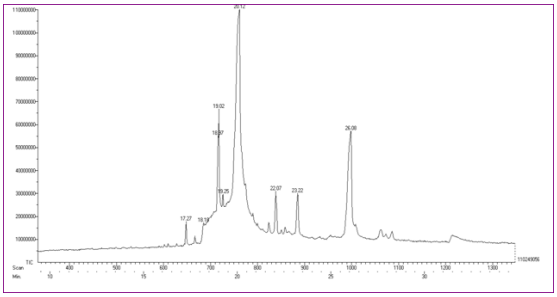

Figure 6: Chromatogram of acetone A.paniculata extract from GC-MS analysis.

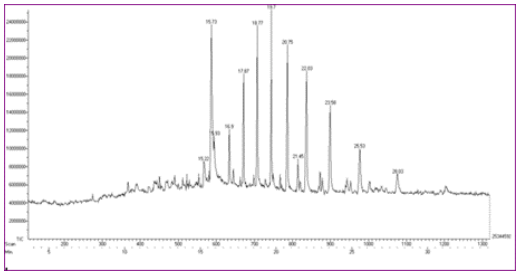

Figure 7: Chromatogram of acetone A.lanata extract from GC-MS analysis

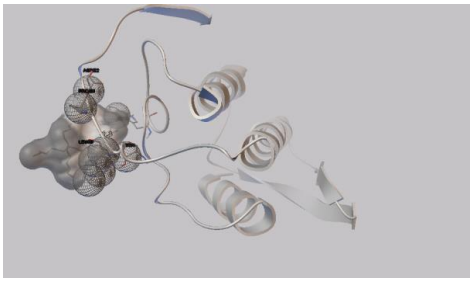

Figure 8: Interaction of Hexadecanoic acid, 1-(hydroxymethyl)- 1,2 ethanediyl ester

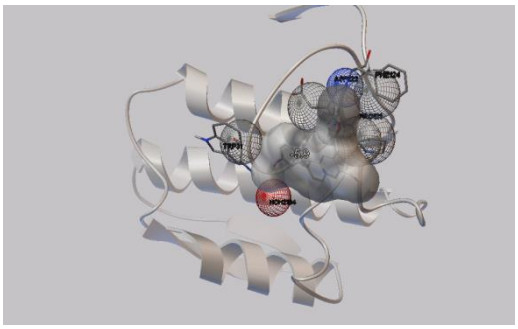

Figure 9: Interaction of Corynan-17.ol.18.19.didehydro-10-methoxy-. Acetate

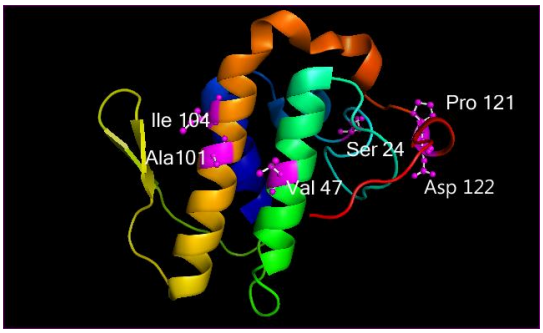

Figure 10: Important Interactive residues of PLA2 receptor with the compounds from the plant extract.

DISCUSSION

In this study we have taken *Andrographis paniculata* (Acanthaceae)and *Aerva lanata* (Amaranthaceae)plants and studied its response on *Naja naja* venom.There were several animal studies on plants like *H. indicus, C.aromatica, A.indica, A.paniculata, C.zedoaria, A.indica, P.indica, E.officinalis, V,negundo, C.aromatica salisb, C.zedoaria rox, Annona senealensis,etc*[22]. There was also evidence that the extracts of the medicinal plants have inhibited the PLA2 enzyme. About 45µl of *Ruta graveolens*[23],0.13mg of *Mimosa pudica*[24], 50µg of *Aristolochia indica*[25], 1.2mg of *Vitex negundo*[26]plant extract is used against *Naja Naja* venom. Our experiment on PLA2 activity showed that 10µl of A.lanata extract, and 20 µl of A.paniculata extract were able to inhibit the activity of *Naja naja* venom which is effective than other plant extracts. Our procoagulant study shows that 30µl of *A.lanata* extract, and 40 µl of *A.paniculata* extract were able to inhibit four times MCD coagulation of *Naja naja* venom. In contrast to our studies, 30µl of *Ruta graveolens*-ethanol extract and 40µl of *Ruta graveolens*- acetone extract neutralized three times *Naja naja* venom MCD value. In contrast to our results on lytic activity alcoholic extract of *C.viscosum* against *Naja naja* venom failed to inhibit haemolysis between concentrations 20-100 µg/ml due to instability of membrane proteins by the plant extract[21]. At the concentrations of 10 µg/ml *A.paniculata, C.magna, G.superba* and *H.javanica* showed 50.99%, 60.89%, 60.60% and 74.21% lysis[27]. 30µl of A.lanata extract and 40 µl of A.paniculata were able to inhibit 50% against *Naja naja* venom.The molecular structure of p-coumaric acid, resveratrol, spermidine, corticosterone and gramine derivative interacted with PLA2 complex forming different binding pattern. The first three molecules interacted directly to the His48, Asp49 residues in innermost cleft of the PLA2 molecule resulting in water molecule interaction. Later, two molecules forms similar binding pattern without water molecule interaction because inability to slide deeply through the cleft of PLA2.The studies on phytochemical from *Aerva lanata* and *Andrographispaniculata*Ile104, Ala101Val 47, Phe 46, Asp122 and Pro121forms the active binding site in α- helix 3 and 4. On analyzing the secondary structure mutational studies will give an insight view on the compounds isolated from the different plants.

The medicinal plants of India are highly explored for effective remedial system of venomous snake bite. Nowadays biological plant extract of traditional plants are used for designing of new active compounds. The present study involving different

medicianl plants like *Andrographis paniculata* and *Aerva lanata* against *Naja naja* venom were analysed. The *invitro* analysis of acetone plant extract estimated value consisting 40 µl of *Andrographis paniculata* and 30 µl *Aerva lanata* are effective in neutralization of *Naja naja* venom. The molecular docking analysis of plant compounds against PLA2 molecule shows the effective binding site in the C-terminal end (Ile104, Ala101), α-helix 3 (Val 47, Phe 46) and α- helix 4 (Asp122, Pro121). The research work shows that *Aerva lanata* could be an effective treatment intreating snake bite in India.

ABBREVIATIONS

Nm	Nanometre
mm	Millimetre
m	Metre
TLC	Thin Layer Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
WHO	World Health Organisation
PLA2	Phospholipase 2
ASV	Anti Snake Venom
R_f	Retention Factor
eV	Electron Volt
PBS	Phosphate Buffer Saline
rpm	Rotations per minute
min	Minutes
MCD	Minimum Coagulant Dose
MIHD	Minimum Indirect Inhibitory Concentration
HRBC	Human Red Blood Cells
VDW	Van Der Waals
SDF	Structure Data File
PDBQT	Protein Data Bank Partial Charge & Atom Type
PDB	Protein Data Bank

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REFERENCES

1. Anita, K., Kishor Shivaji, C. VKG. Medicinal plants active against snake Envenomation. International Journal of Research in Ayurveda and Pharmacy. 2012;3(3):363–8.
2. Bijayeeni, M., David, A.W., Wilson, S., Prakash, B., Neeraj, D., Raju, M.J., Peter, S. R., Kaushik, M., Romulus, W., Prabhat J. Snakebite Mortality in India: A Nationally Representative Mortality Survey. Public Library of Science neglected Topical Diseases. 2011;5(4):1018.
3. Rafael SF. Effect of Mikania glomerata (Asteraceae) leaf extract combined with anti-venom serum on experimental Crotalus durissus (Squamata: Viperidae) envenomation in rats. International Journal of Tropical Biology and Conservation. 2000;57(4):929–37.
4. Huang, L.F., Zheng, J.B., Xu, Y., Song, H.T., Yu CX. . A snake venom phospholipase A2 with high affinity for muscarinic acetylcholine receptors acts on guinea pig ileum. Toxicon. 2008;1(2):317–51.
5. Giachetti, D., Monti T. Medicinal plants in phytotherapy. Annali dell'Istituto Superiore Di Sanita2004;41(1):17–22.
6. Dubey, N.K., Kumar, R., Tripathi P. Global Promotion of Herbal Medicine: India's Opportunities. Current Sciences. 2004;86(1):37–41.
7. Vishwanath, B.S., Gowda TV. Interaction of Aristolochic acid with Vipera russelli Phospholipase A2, its effect on enzymatic and pathological activities. Toxicon. 1987;29(9):929–37.
8. Nazimuddin, S.K., Ramaswamy, S., Kameswaran L. Effect of Andrographis paniculata on snake venom induced death and its mechanism. Indian Journal of Pharmaceutical Sciences. 1978;40(4):132–3.
9. Aguiyi, J.C., Guerranti, R., Pagani, R., Marinello E. Blood chemistry of rats pretreated with Mucuna pruriens seed aqueous extract MP101UJ after Echis carinatus venom challenge. Phytotherepy Research. 2001;15:712–4.
10. Mahanta, M., Mukherjee, A.K. Neutralization of lethality, myotoxicity and toxic enzymes of Naja kaouthia venom by Mimosa pudica root extracts. Journal of Ethnopharmacology. 2001;75:55–60.
11. Chatterjee, I., Chakravarty, A.K., Gomes A. Daboia russellii and Naja kaouthia venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla Hemidesmus indicus R.Br. Journal of Ethnopharmacol. 2006;106:38–43.
12. Saurabh, S., Pradeep, S., Garima, M., Jha, K.K., Khosa RL. Achyranthes aspera-An important medicinal plant: A review. Journal of Natural Product and Plant Resources. 2011;1(1):1–14.
13. Manokaran, S., Jaswanth, A., Sengottuvelu, S., Nandhakumar, J., Duraisamy, R., Karthikeyan, D., Mallegaswari R. Hepatoprotective activity of Aerva lanata Linn. against paracetamol induced hepatotoxicity in rats. Research Journal of Pharmacy and Technology. 2008;1(1):398–400.
14. Kalaiselvan, A., Gokulakrishnan, K., Anand T. Gas chromatography-Mass spectrum analysis of bioactive components of the ethanol extract of Andrographis paniculata. Journal of Pharmaceutical and Biomedical Sciences. 2012;20:99–104.
15. Mohanta, T.K., Patra JK. Evaluation of Antimicrobial Activity and Phytochemical Screening of oils and Nuts of Semicarpus anacardium. Scientific Research and Essays. 2007;11(2):486–90.
16. Chakraborty, D.D., Ravi, V., Chakraborty P. Phytochemical evaluation and TLC protocol of various extracts of Bombax ceiba Linn.International Journal of Pharmaceutical Sciences and Resources. 2010;8(1):66–73.
17. Trott, O., Olsen AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry. 2010;31(2):455–61.
18. Gutierrez, J.M., Avila, C., Rojas, E., Cerdas L. An alternative in vitro method for testing the potency of the polyvalent antivenom produced in Costa Rica. Toxicon. 1988;26:411–3.
19. Meenatchisundaram, S., Micharl A. Antitoxin activity of Mucuna pruriens aqueous extracts against cobra venom and krait venom by invivo and invitro methods.Indian Journal of Science and Technology. 2010;2(1):870–4.
20. Geng Y, Sun F, Ma Y, Deng L, Lü J, Li T, et al. Serum metabolomics analysis on benign prostate hyperplasia in mice based on liquid chromatography-mass spectrometry. Chinese Journal of Chromatography. 2014;32(12):1301–5.
21. Richard Lobo, Punitha, I.S.R., Rajendran, K., Arun Shirwaikar AS. Preliminary study on the Antisnake Venom Activity of Alcoholic extract of Cleodrendrum viscosum (Vent.) in Naja naja venom. Natural Product Sciences. 2006;12(3):153–6.
22. Adzu, B., Abubakar, M.S., Izebe, K.S., Akumka, D.D., Gamaniel KS. Effect of Annona Senegalensis rootbark extracts on Naja nigricotlis nigricotlis venom in rats. Journal of Ethnopharmacology. 2005;96:507–13.
23. Ebenezu Samuel King, J., Balasubramanian M. Identification and characterization of Bioactive compounds from Ruta

graveolens L. Leaf extract against Naja naja venom. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2015;6(4):475–82.
24. Meenatchisundaram, S., Parameshwari, G., Michael A. Studies on antivenom activity of Andrographis paniculata and Aristolochia indica plant extracts against Daboia russelli venom by in vivo and in vitro methods. Indian Journal of Science and Technology. 2009;2(4):76–9.
25. Mukherjee, A.K., Doley, R., Saikia D. Isolation of a snake venom phospholipaseA2 (PLA2) inhibitor (AIPLAI) from leaves of Azadirachta indica (neem): Mechanism of PLA2 inhibition by AIPLAI invitro condition. Toxicon. 2008;51:1548–53.
26. Brindha, D., Santhoshkumar, M., Krupa S. In vitro antivenom and antioxidant potential of Vitex negundo leaves (green and blue) against Russel's viper (Daboia russeli) and Indian cobra (Naja naja) venom. European Journal of Experimental Biology. 2014;4(4):207–19.
27. Chidambaram Kumarappen, Albert Jaswanth KK. Antihemolytic and snake venom neutralizing effect of some medicinal plants. Asian Pacific Journal of Tropical Medicine. 2011;743–7.

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