Biocatalysis and Agricultural Biotechnology 18 (2019) 101058

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Molecular interaction of phytochemicals with snake venom: Phytochemicals of *Andrographis paniculata* inhibits phospholipase A2 of Russell's viper (Daboia russelli)

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ARTICLE INFO

Keywords:
Metapocket
AutoDock
Anti-oxidant
Protein-ligand interaction
DPPH
Deoxyandrographolide
Andrograpanin

ABSTRACT

Andrographis paniculata belonging to Acanthaceae family is an annual herb, traditionally used for venomous bites in various parts of Asia. The plant has various phytochemicals with numerous biological properties which are chemically well characterized and available in the database out of which 22 phytochemicals was used for the study. Our study focuses on finding a lead phytochemical by employing various *in-silico* methods (Metapocket and Autodock) for the inhibition of venomous protein, namely phospholipase A2. Phospholipase A2 (PLA A2) is conserved in snakes and also considered to be the major toxin possessing equally lethal effect, neurotoxic and myotoxic. Though the PLA A2 three dimensional structure is available, finding the active motif of PLA A2 will make the molecular interaction (docking) studies accurate. Hence, MetaPocket, a tool used for finding the active site as it encompass the results of eight independent pocket site prediction methods. The ability of phytochemical to bind the predicted active site of PLA A2 is carried out with the widely used simulation method, Autodock. Results provided a strong evidence for the presence of multiple putative anti-venom (inhibitor of PLA A2) phytochemicals in *A. paniculata* (esp., Deoxyandrographolide, Andrograpanin, etc.) and *A. paniculata* is also found to be a potent free radical scavenger (anti-oxidant) assessed by DPPH in *in-vitro* condition. Oxidative stress is of major complication reported in snake bitten patient's further worsening the clinical condition and requiring an additional treatment with anti-oxidants in long-term. In conclusion, the phytochemicals of *A. paniculata* is found to be a potent inhibitor of natural origin targeting PLA A2 to neutralize snake venom.

1. Introduction

Andrographis paniculata is an annual herb of Acanthaceae family widely found in many Asian countries including India, Srilanka, Malaysia, Indonesia; and also in West Indies and some parts of America. *A. paniculata* is known as “king of bitters” (Nyeem et al., 2017), colloquially called as *kalmegh* in India, *chaunxin lian* in China, *Hempedu bumi* in Malaysia (Kumar et al., 2004).*A. paniculata* is short branched erect herb and fragile in texture (Niranjan et al., 2010). *A. paniculata* is known for its medicinal property. For centuries the whole plant has been used by the traditional practitioner for treating various ailments like respiratory diseases, dysentery, skin infection, sore throat, fever, diarrhea, and urinary tract infection. In traditional Chinese medicine the plant is used for regulating body temperature during infection (Joselin and Jeeva, 2014). *A. paniculata* have been scientifically evaluated and found to have anti-bacterial, antioxidant, anti-inflammatory, anti-cancer, antiviral and anti-fungal, anti-malarial property, immunomodulatory, hepatoprotective activity, cytotoxicity, anti-diabetic, anti-angiogenic, liver enzyme modulatory property (Anju et al., 2012; Sagadevan et al., 2015). *A. paniculata* is acknowledged as king of bitters as its bitterness is not comparable with any other plants. The bitter *A. paniculata* is also known for treatment of venomous bites by the tribes and traditional medicine practitioners (Gomes et al., 2010). Few scientific reports also support the antivenom property of *A. paniculata*, however the phytochemical responsible and the mechanism by which it neutralizes the venom is not uncovered. Since *A. paniculata* has numerous phytochemicals which may require longer time and expensive to find specific biological function(s) of each phytochemical. Bioinformatics tool provide a wide array of mathematical methods formulated based on the experimental evidence which helps in ascertaining the interactions between molecules. Henceforth, the interaction of snake venom with different phytochemicals of *A. paniculata* was studied.

PLA A2 is present in almost all snake venom and rich in Russell

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https://doi.org/10.1016/j.bcab.2019.101058
Received 12 December 2018; Received in revised form 12 February 2019; Accepted 23 February 2019
Available online 02 March 2019
1878-8181/

viper being one of the big four snakes of India causing high mortality rate (Mohapatra et al., 2011). PLA A2 hydrolyzes phospholipid having unsaturated fatty acid at sn-2 position of ester bond leading to lysophospholipid and unsaturated fatty acid generation in a calcium dependent manner. This results in cell membrane physical property change, activation of downstream signal transduction pathway which in turn affects the cell homeostasis all over body (Gasanov et al., 2014). PLA A2 is precursor for eicosanoids which mediates various inflammatory processes. Apart from hydrolyses of phospholipid PLA A2 also has various other pathophysiological effects such as neurotoxic, myotoxicity, anticoagulation, cardiotoxic, oedematogenic and inflammation in victims (Dias et al., 2019). Since PLA A2 being a small enzyme composed of 120–125 amino acids residues stabilized by seven disulfide bridges (Fatima and Fatan, 2014) is a potent target for the pharmacological interventions. Our study involves computational approach for finding the best PLA A2 inhibitor from selected phytochemicals of *A. paniculata*.

2. Material and methods

2.1. Ligand preparation

The structure of 22 phytochemicals from *A. paniculata* and a known PLA A2 inhibitor, Anagrelide which is used for treating thrombocytosis are obtained from PubChem (Bolton et al., 2008). The details of the ligand of interest are tabulated in Table 1 and its structure is presented in Fig. 1.

2.2. Protein preparation

The target protein Phospholipase A2 of Russell's viper was obtained from protein databank (PDB ID: 2PYC). The structure of PLA A2 used for the study is shown in Fig. 2.

Table 1
List of selected phytochemicals in *A. paniculata* with PubChem ID.

S·No	Phytochemical Name	Pubchem Id	Reference
1	Neoandrographolide	9848024	Tan et al. (2016)
2	Andrographidine C	5318484	Tan et al. (2016)
3	Andrographidine E	5318498	Tan et al. (2016)
4	Andrographoside	6439612	Rajani et al. (2000)
5	14-acetylandrgrapholide	73353957	Duke (1992)
6	Andropanoside	44575270	Hapuarachchi et al. (2013)
7	Andrographidine A	13963762	Tan et al. (2016)
8	Andrographiside	44593583	Hapuarachchi et al. (2013)
9	Chlorogenic acid	1794427	Low et al. (2015)
10	Deoxyandrographolide	21679042	Tan et al. (2016)
11	14-deoxy-11-oxoandrographolide	101593061	Duke (1992)
12	Andrograhin	5318506	Niranjan et al. (2010)
13	14-deoxy-11,12dehydroandrographolide	5708351	Cai et al. (2015)
14	Andrographolide	5318517	Tan et al. (2016)
15	Isoandrographolide	101563021	Tan et al. (2016)
16	Andropanolide	7067324	Pramanick et al. (2006)
17	Andrograpanin	11666871	Tan et al. (2016)
18	Myristic acid	11005	Joselin and Jeeva (2014)
19	Paniculide A	11821485	Duke (1992)
20	Paniculide C	101289824	Duke (1992)
21	Paniculide B	101289823	Duke (1992)
22	Bisandrographolide	12000062	Chen et al. (2006)
23	Anagrelide	2182	Domi et al. (2017)

2.3. Binding pocket analysis

To identify an active site/pocket in PLA A2 a tool Metapocket was used. “Metapocket 2.0” is a meta server created primarily to identify ligand binding sites on the exposed regions of peptides and protein. Metapocket is a mathematical method, which analyses the results produced by autonomous binding sites predictor tools (LIGSITE^{CS}, PASS, Q-SiteFinder, SURFNET, Fpocket, GHECOM, ConCavity, and POCASA) in order to improve the success rate of prediction. The z-score produced by these autonomous tools for each pocket site predicted is accounted in producing the total z-sore. The pocket sites will be ascertained based on the spatial similarity and all the final clusters will be ranked by the total z-score calculated by metapocket. Finally, functional residues around the identified meta-pocket site will be highlighted and all the results of metapocket will be presented in the form of table.

2.4. Molecular docking analysis

In order to find the binding possibility of chosen ligands with the active site of PLA A2, *insilico* method autodock is used. Autodock tool is commonly used to predict the binding efficiency of a ligand to a peptide/protein by using a combination of mathematical calculations and algorithms. AutoDock 4 is the advanced version of AutoDock where newly added feature is flexibility of sidechains in the protein during the process of ligand docking. According to the AutoDock licenser, it has a free-energy scoring function based on a linear regression analysis, the AMBER force field, and an even larger set of diverse protein-ligand complexes with known inhibition constants than the earlier version. In AutoDock 4, polar/non-polar hydrogen bond, Gasteiger/kollman charges are added to the protein followed by saving ligand and target protein in pdbqt format. Binding energy, inhibition constant is analyzed and produced as docking output (Morris et al., 1999).

2.5. Visualization

Biovia Discovery Studio Visualizer is a free visualization and analysis suite, has various features namely visualization, macromolecule design, ligand based design, structure-based design. An interactive 3D simulation tool to visualize and analyze small molecule, protein, nucleic acid, and their crystal structure etc., BIOVIA Discovery studio 2017R2 is used for the current study to visualize and analyze docked ligand with protein and figures presented in the manuscript.

2.6. Free radical scavenging assay using DPPH

Commercially available dried plant was pulverized tofine powder by using blender. 50 g of powder was extractedwith 70% methanol by maceration (Azwanida, 2015). The whole extract was filtered using Whatmann filter paper no.1 and the methanol was evaporated to getcrude extract. The free radical scavenging potential of *A. paniculata* was measured using a stable free radical DPPH (1, 1- diphenyl 2-picrylhyorazyl) according to the method described by Brand-Williams et al. (1995) with slight modifications. 0.1 mM of DPPH was incubated with methanolic extract of *A. paniculata* at various concentrations (50–250 µg/ml) under the dark condition for 20 min. Simultaneously tubes for blank (only ethanol), sample blank (*A.paniculata* extract at various concentration except DPPH) and control (DPPH solution) was also maintained. The assay was performed in triplicate and the decrease in absorbance was measured at 517 nm using UV–Vis spectrophotometer. The free radical scavenging potency in terms of decrease in DPPH colors was calculated using the following formula.

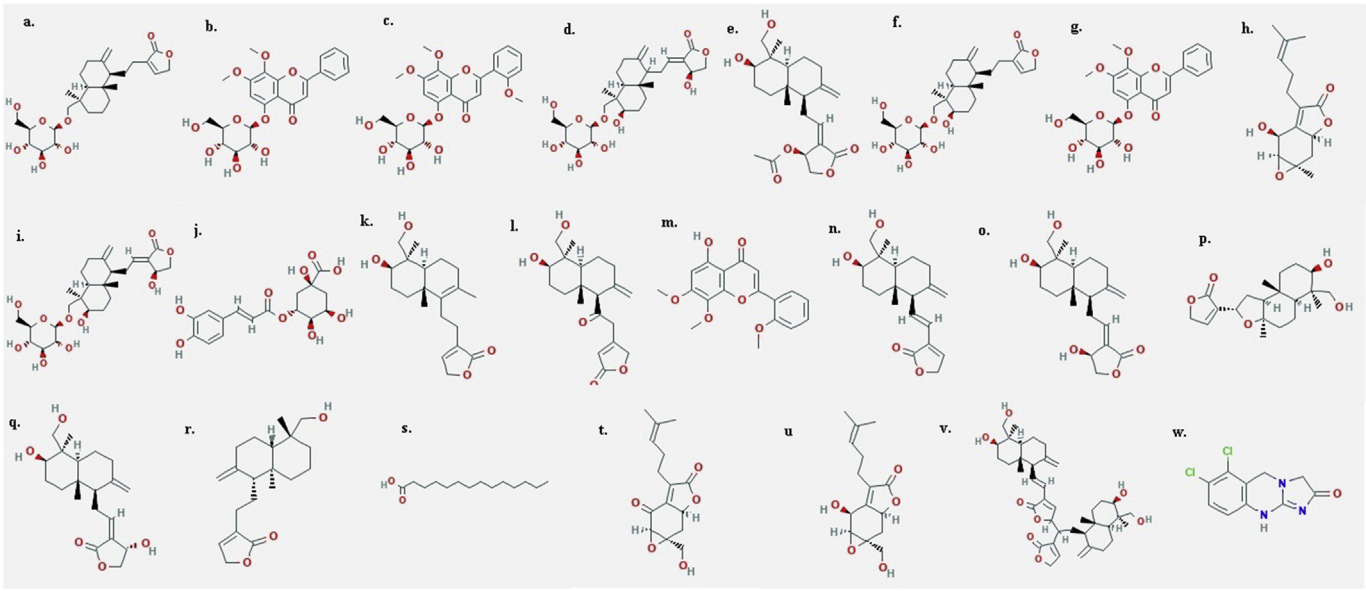

Fig. 1. Structure of phytochemicalsand standard drug The
two dimensional structure of phytochemicalsand standard drug used for the study which is available in PubChem are presented. a.Neoandrographolide, b. Andrographidine C, c. Andrographidine E, d. Andrographoside, e. 14-Acetylandrographolide, f. Andropanoside, g. Andrographidine A, h. Paniculide A, i. Andrographiside, j. Chlorogenic acid, k. Deoxyandrographolide, l. 14-Deoxy-11-oxoandrographolide, m. Andrographin, n. 14-deoxy-11 12dehydroandrographolide, o.Andrographolide, p. Ioandrographolide, q. Andropanolide, r. Andrograpanin, s. Myristic acid, t. Paniculide C, u. Paniculide B, v. Bisandrographolide, w. Anagrelide.

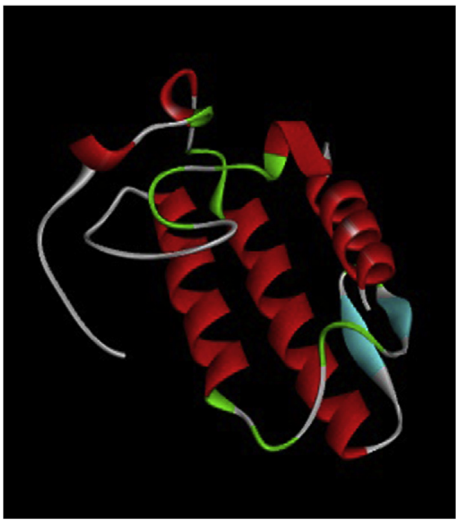

Fig. 2. Three dimensional structure of Phospholipase A2.
The 3D structure of Phospholipase A2 of the snake Russell's viper used for the analysis is presented and which is available in protein data bank (PDB ID: 2PYC).

$$\text{Inhibition } \% = \frac{\text{Absorbance recorded for control} - \text{absorbance recorded for samples}}{\text{Absorbance recorded for control}} \times 100$$

3. Results and discussion

Snakebite became a worldwide threat killing nearly 81,000–138,000 people and 400,000 being permanently disabled every year (WHO, 2018). This huge number of affected individual is considered a red alert by WHO. The majority of affected person are associated with agriculture hence a special consideration is been given over other neglected diseases as snake bite is categorized under neglected diseases previously by WHO. Snake venom being highly heterogeneous and proteinous makes it difficult to treat. Snake venom consists of a number of toxic proteins and polypeptides like phospholipase A2, snake venom metalloproteinase, serine protease, and three finger toxin (Tasoulis and Isbister, 2017) and some report presence of DNA in snake venom (Pook and McEwing, 2005). Many antibody-based antivenoms (immunotherapy) has been developed for snakebite treatment although these antivenoms are effective against systemic envenoming but ineffective on local envenoming effects that occur soon after snake bite leading to paralysis and permanent deformity (Lalloo and Theakston, 2003).

Moreover, the cost and ethical issue involved in producing and maintenance of venom-specific antibody makes it unreachable for many. The lack of resources for antibody-based treatment (immunotherapy) redirects the researchers back to plant-based medicines used traditionally in various parts of the world. Scientific literature has produced convincing evidence for traditional medicine (Upasani et al., 2018). *A. paniculata* is one such plant which has numerous medicinal claims.

For treating snakebite, *A. paniculata* has been used in various forms by local tribes of India; tribes of Andhra Pradesh uses whole seed powder and tribes of central India consumes plant paste along with mustard oil (Dey and JithendraNath, 2012). Several scientific studies have also shown protective effect of *A. paniculata* against various snake venom. *In vivo* studies revealed that 0.15 mg of *A. paniculata* extract was sufficient to completely neutralize the lethal dose (LD50) of Russell viper (Meenatchshisundaram et al., 2009) and also the toxicity produced by Cobra and Viper venom in both *in vivo* and *in vitro* (Alam, 2014). Neurotoxic effects *Naja naja* snake venom significantly nullified by the *A. paniculata* methanol extract*in vivo* and *in vitro* studies (Gopi et al., 2011). Alcoholic extract of *A. paniculata* considerably neutralized the Naja nagricollis and stabilized membrane against the hyposaline induced damage in RBCs (Kumarapppan et al., 2011). There exists strong scientific evidence confirming the traditional use of *A. paniculata* suggesting the presence of pharmaceutically valuable lead molecule (s). Present study was carried out to identify the phytochemical which directly inhibits venomous enzyme. In search of such lead molecule, various suitable *insilico* tools were used and the results are discussed.

Table 2
Metapocket result for PLA A2.

ATOM		Binding Site	Software	Ranking	x	y	z	ranking	z-score
ATOM	1	C3	CON	1	2.618	18.188	5.928	1	1.00
ATOM	2	C3	FPK	1	5.151	19.918	6.674	1	2.45
ATOM	3	C3	GHE	1	4.003	19.814	6.017	1	2.17
ATOM	4	C3	LCS	1	3.226	18.961	5.981	1	3.19
ATOM	5	C3	PAS	1	2.209	20.624	4.029	2	0.18
ATOM	6	C3	SFN	1	5.565	17.527	5.144	1	5.58
ATOM	7	C3	GHE	2	−5.139	23.383	10.812	2	0.71
ATOM	8	C3	LCS	2	−5.395	23.012	10.572	2	1.06
ATOM	9	C3	PAS	2	−5.063	24.429	11.318	1	1.87
ATOM	10	C3	SFN	3	7.458	20.156	−13.261	0	0.37
TER									
ATOM	1	C3	MPT	1	3.795	19.172	5.629	6	14.57
ATOM	2	C3	MPT	2	−5.199	23.608	10.901	3	3.64
ATOM	3	C3	MPT	3	7.458	20.156	−13.261	1	0.37

Table 3
Binding affinity of phytochemicals from Andrographis paniculata with snake venom Phospholipase A2.

S·NO	Phytochemical name	Binding Energy (kcal/mol)	Inhibition Constant (Ki)	Binding residue
1	Deoxyandrographolide	−8.48	611.02 nM	UNK0:H, LYS69:HZ3
2	Andrograpanin	−8.32	791.42 nM	UNK0:H, GLY33:HN
3	14-Acetylandrographolide	−8.12	1.12 μM	UNK0:H, HIS48:HDI, LYS69:HZ3
4	Isoandrographolide	−8.12	1.11 μM	UNK0:H, LYS69:HZ3
5	14-Deoxy −11,12dehydroandrographolide	−8.01	1.34 μM	UNK0:H
6	14-Deoxy-11-oxoandrographolide	−7.99	1.39 μM	GLY30:HN, LYS69:HZ3
7	Bisandrographolide	−7.97	1.45 μM	LYS69:HZ3
8	Andrographin	−7.54	2.99 μM	UNK0:H
9	Andrographolide	−7.44	3.51 μM	UNK0:H, TRP31:HN
10	Paniculide C	−7.36	4.05 μM	HIS48:HDI, LYS69:HZ3
11	Andropanolide	−7.3	4.48 μM	UNK0:H, TRY31:HN, GLY32:HN, LYS34:HN
12	Paniculide B	−7.13	5.98 μM	UNK0:H, HIS48:HDI
13	Andrographidine E	−7.09	1.39 μM	UNK0:H, UNK0:H, TRY31:HN, HIS48:HDI, LYS69:HZ3
14	Chlorogenic acid	−7.0	7.44 μM	UNK0:H, UNK0:H, UNK0:H, LYS69:HZ3
15	Paniculide A	−6.96	7.92 μM	UNK0:H, HIS48:HDI
16	Andrographidine C	−6.92	8.5 μM	UNK0:H, GLY30:HN, HIS48:HDI
17	Andrographidine A	−6.62	14.07 μM	UNK0:H, UNK0:H, HIS48:HDI, LYS69:HZ3
18	Neoandrographolide	−6.39	20.58 μM	GLY30:HN, HIS48:HDI, ASN67:HD21
19	Andrographoside	−5.84	52.04 μM	UNK0:H, GLY30:HN, HIS48:HDI
20	Andropanoside	−5.52	89.48 μM	LYS69:HZ3
21	Myristic acid	−5.52	92.13 μM	LYS69:HZ3
22	Andrographiside	−4.3	703.46 μM	GLY30:HN
23	***Anagrelide***	−7.01	7.3 μM	UNK0:H, LYS69:HZ3

S.no 1-22 Phytochemicals of *Andrographis paniculata*, S.no:23 Anagrelide a known inhibitor of PLA A2 is used as a standard drug. Binding energy is the totality of intermolecular energy and torsional free-energy penalty; Inhibition constant is the required concentration for producing half maximum inhibition by an inhibitor. Inhibition constant is a software output. kcal/mol-kilocalorie per mole, Ki-inhibitor constant, μM-micromolar, nM-nanomolar.

The results of the metapocket, tool used to predict possible binding pocketsin proteins are summarized in Table 2. Top 3 pockets as predicted by 8 tools are highlighted along with the calculated z sore. From the metapocketresult, the top ranked pocket has been used for further analysis.

To identify the phytochemical(s) which has a strong affinity towards one of the crucial component of venom mediated toxicological effect, docking was performed. The enzyme PLA A2 found in most of the snake venom is chosen for the study. Around 35% of whole snake venom is composed of PLA_2 esp.,in russelii species referred as one among India's big 4 (Kalita et al., 2017; Tan et al., 2015). There are numerous (51 entries) protein structure available in the protein data bank (PDB) for PLA A2, of which the crystal structure with the PDB ID 2PYC is been selected as it is from the snake Russell's viper. Further, 2PYC crystal structure of a monomeric phospholipase A2 from Russell's viper at 1.5A resolution has high structure resolution than 2PVT crystal structure of a isoform of phospholipase A2 from Russell'viper at 2.1A resolution. In a previous study, Hexadecanoic, 1-(hydroxymethyl)- 1,2 ethanediyl ester of *A. paniculata* found to interact with the residues (TRP31, ASP122, PRO121, SER24, LEU19) of 2PYC with binding energy −5.3 (Mohanapriya et al., 2017). Hence the crystal structure of PLA A2 available with the PDB ID: 2YPC is used for the current investigation.

The results of docking studies using the AutoDock tool is summarized in Table 3. All the selected phytochemical are found to have interaction with the protein PLA A2, however, the degree of the affinity in terms of binding energy was found to be varied. Based on the binding affinity the phytochemical are arranged in Table 3, the phytochemical having the highest affinity is given in the order; Deoxyandrographolide > Andrograpanin > 14-Acetylandrographolide > Isoandrographolideis shown in Fig. 3 along with their binding residue.

The structure of PLA A2 is simple possessing a α-helix, β-sheet, catalytic site and calcium-binding site. PLA A2 is 125 amino acid containing protein, of which HIS48, TYR52 and ASP99 are considered as catalytically crucial residues and the residues in 53 and 76 is responsible for the anticoagulation (Kini, 2005; Komori et al., 2012). The phytochemicals of *A. paniculata* that are found to interact with the HIS48 at the catalytic site are 14-Acetylandrographolide, Paniculide C, Paniculide B, Andrographidine E, Paniculide A, Andrographidine C, Andrographidine A, Neoandrographolide and Andrographoside.

Previous studies on N-terminal region of 26 PLA_2 (toxic) and non-

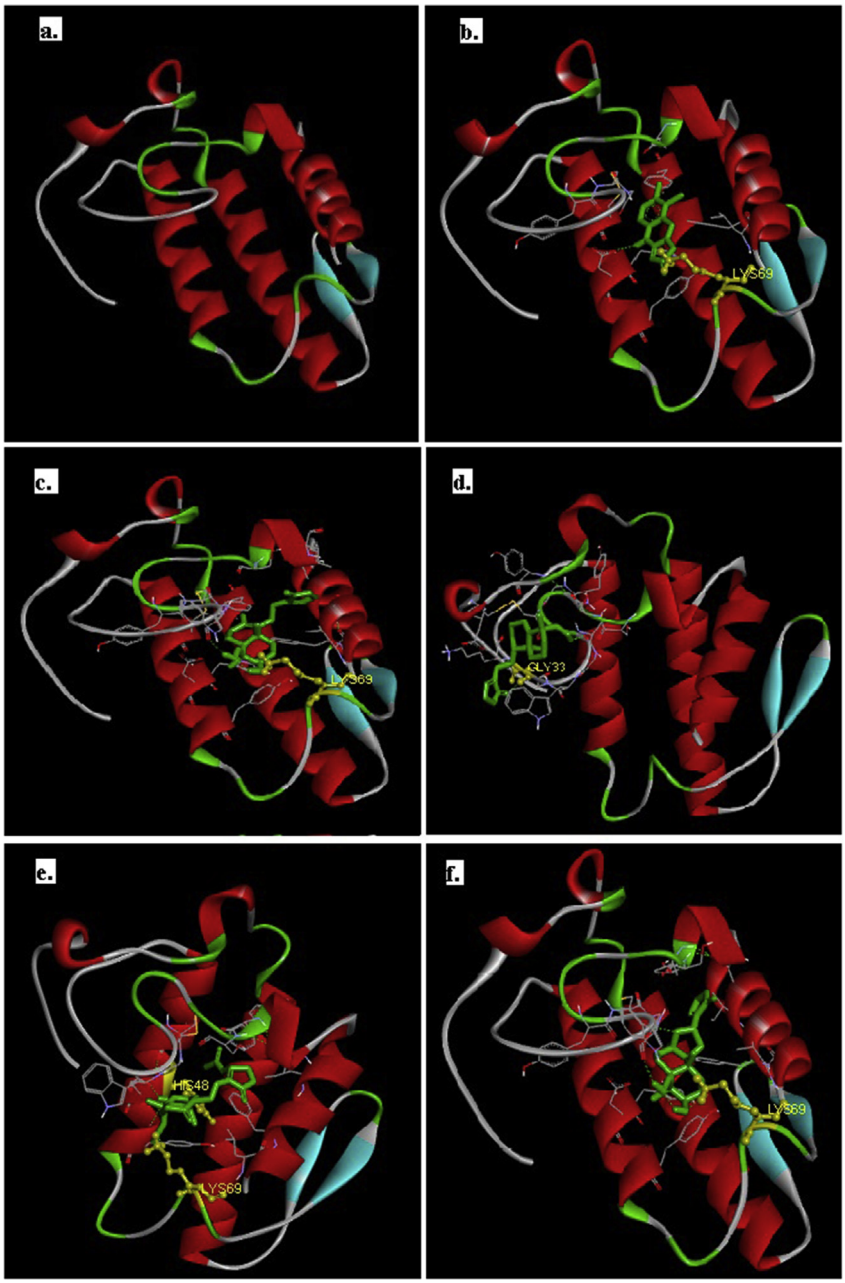

Fig. 3. PLA A2 with selected ligands.
The PLAA2 with respective ligands highlighting the interacting residue is presented. a. PLA A2, b. PLA A2 docked with known inhibitor Anagrelide, c. PLA A2 docked with Deoxyandrographolide, d. PLA A2 docked with andrograpanin, e PLA A2 docked with 14-Acetylandrographolide, f. PLA A2 docked with Isoandrographolide.

neurotoxic enzyme, and found that neurotoxicity is associated with neutral residue 6 and charged residue 6 with non-neurotoxic (Tsai and Tzeng, 1992). In our result, none of the compound was found to interact with the residue in 6th position. Basic residues 59–73, 97 or 98 at N-terminus and 1–7, 64–81 and 97–109 residues of nearby hydrophobic region of PLA A2 (presynaptically toxic) collectively responsible for the toxicity enabling them to bind with specific membrane receptor and neurotransmission reduction at the initial phase (Tsai et al., 1987). The Met8, TRP31 and TRP69 are sensitive for enzymatic activity of PLA A2 of Australian king brown snake (Takasaki et al., 1990). In another report, Taiwan Russell's viper (Vipera russelli formosensis) venom's residues 6,12, 76–81 and 119–125 were responsible for neurotoxicity. Numerous phytochemical studied were found to have a strong interaction with the mentioned neurotoxic regions. Neurotoxic PLA A2 possessa neutral charged residue at position 6 like ASN, ALA or GLY and not having LYS at 12th position and amino acid residues at the regions spanning 76–81, 119 and 125 are basic residues (Wang et al., 1992). When the residue at position 6 replaced with charged residue resulted in complete halt of substrate (transition state analog) entrance towards the catalytic site (HIS48, TRY52, ASP49, ASP99) of PLA A2. This substitution has resulted in loss of enzyme specificity (Wery et al., 1991). However there is no notable interaction of the phytochemicals of *A. paniculata* on these regions chaining the charge of the residue.

Mutational analysis of residues, GLU-56 and LYS-69 showed a loss of substrate preference towards phosphatidic acid during hydrolysis (Snitko et al., 1997). In addition, experimental and modeling studies have shown the importance of LYS69 in the active site (Nirmal et al., 2008). Out of 22 phytochemicals, 11 were able to get docked with the LYS 69 residues owing to a big change in the substrate preference.

Quercetin-3-O-rhamnoside (isolated from *Euphorbia hirta*) a

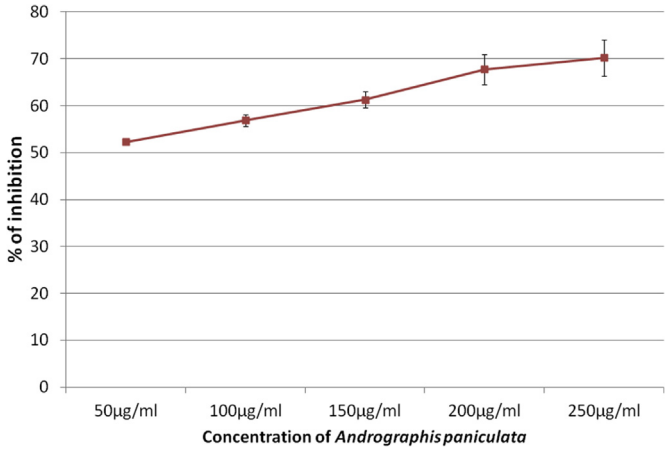

Fig. 4. Peptide sequence of snake venom mapped with biological activity based on existing literature and site of interaction of phytochemicals of *A. paniculata*.

derivative of Quercetin is found to form four hydrogen bonds with (TYR63, CYS44, TYR27 and ASP48) residues of PLA A2 of Naja naja (high binding energy −8.2 kcal/mol) than quercetin forming only two hydrogen bonds with (HIS47) of PLA A2 of catalytic site (−7.9 kcal/mol binding energy). This might be due to increase in hydrogen bond strength between Quercetin-3-O-rhamnoside and PLA A2. Quercetin-3-O-rhamnoside (QR) along with venom in different ratios (venom: QR) under *in-vitro* conditions found to inhibit various toxicological activities in dose dependent manner (28% casein proteolysis inhibition (1:20 ratio), 93% decrease in hyaluronidase activity at (1:50),reduction in hemolytic activity at (1:20 ratio)). In *in-vivo*, venom injected along with QR in the ratio 1:40 and 1:80 prolonged the survival time by 170 and 302 min compared to control animals injected with venom alone (Gopi et al., 2016). However, in the current study no significant interaction of phytochemicals with the mentioned residues was noted. A schematic presentation of the active sites and other crucial site reported in PLA A2 is presented along with the interacting phytochemical of A. *paniculata* for the better understanding of the results presented in the current study (Fig. 4).

The available treatment for snake venom is immunotherapy which is effective in saving the life of the victim is, however, fails in the prevention of venom-induced chronic complications. The two major such complications are inflammation and oxidative stress. The mechanism by which immunotherapy detoxifies the venom is itself is a pro-inflammatory process leading to the production of several cellular pro-inflammatory markers such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and cyclo-oxygenase (COX)-2 (Santhosh et al., 2013)which is more complicated to address. Oxidative stress is the second major venom-induced chronic complication reported by several clinicians.

There is a shift from oxidative state to oxidative stress state in patients admitted with a venomous bite. A significant increase in oxidative stress index (OSI), oxidant status (TOS) was noted in patients with snake envenomation compared with controls (Zengin al, 2014). Hence an anti-oxidant supplement in addition to immunotherapy has proven to be effective in early immunotherapy as well as in long term treatment. Several antioxidants both synthetic and natural like ascorbic acid, crocin, melatonin are shown to be effective in mitigating venom-induced oxidative damages (Wasim Khan et al., 2016). Results of free

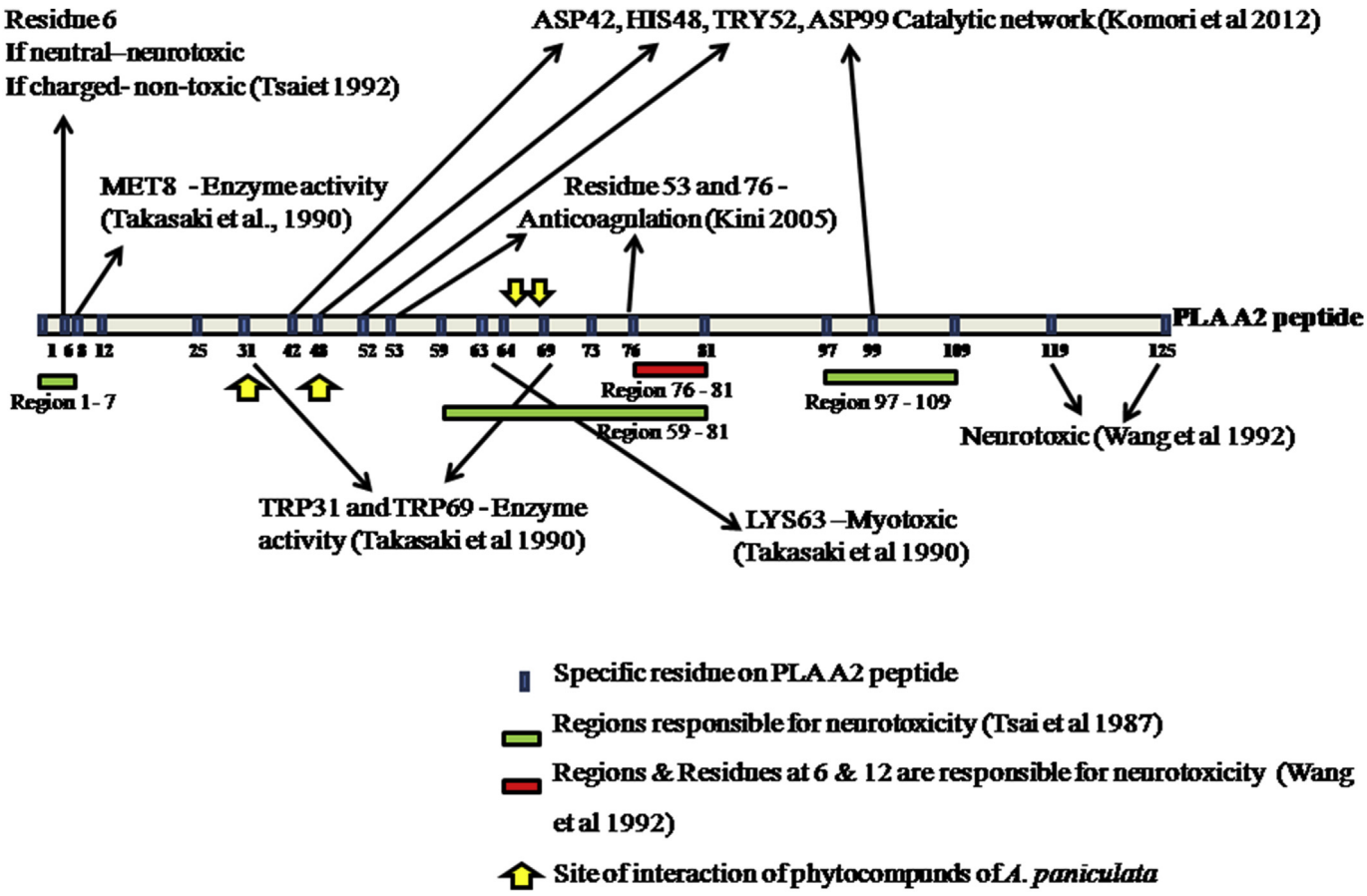

Fig. 5. Free radical scavenging potency of *Andrographis paniculata*.
Free radical scavenging potency in terms of DPPH colour quenching by *Andrographis paniculata* alcoholic extract studied in various concentration and presented as graph (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

radical scavenging assay is represented in Fig. 5. *A. paniculata* at various concentration (50, 100, 150, 200 and 250 μg/ml) showed the free radical scavenging property. A maximum of 70% inhibition has noted a concentration of 250 μg/ml and also a dose-dependent inhibition of free radical production is noted which confers the multiple methods of neutralizing the venom. Firstly by direct inhibition PLA A2 and on other hand by reducing the oxidative stress.

Though plant based drugs are expected to be effective against disease may also pose some adverse effect making it unsuitable for pharmaceutical uses. Hence in order to further validate the medicinal use of *A. paniculata,* a thorough understating of its toxicity is also equally important. In a clinical study conducted with 61 patients, 1200 mg of standardized extract of *A. paniculata* for treating common cold daily basis showed no significant adverse reaction (Hancke et al., 1995). However, in WHO report, a mild gastric discomfort, vomiting, loss of appetite is associated with high dose of *A. paniculata* (WHO, 2002). Likewise, several clinical and pre-clinical studies conducted on evaluating the pharmacological activity of *A. paniculata* didn't showed significant toxicity or mild discomfort (Muangman et al., 1995).

Currently, the only method available of saving human from snake bite is by immunotherapy. Though the treatment of snake bite with immunotherapy is effective; it also pose few limitations such as adverse effects, cost and availability (Ahmed et al., 2008). Loss of crucial hour and lack of treatment attributed to the high mortality rate. Herbal remedies are preferred over immunotherapy due its non-availability and ineffectiveness on account of species specificity (Gupta and Peshin, 2014). A comparative study has been reported in mice exposed to Naja naja snake venom to point the possible use of *A. paniculata* for snake bite. A high dose (2 g/kg) of *A. paniculata* ethanolic extract along with immunotherapy in mice notably increased the mean survival time and mortality rate compared with control mice which received only immunotherapy (Premendran et al., 2011).

The other draw back in using immunotherapy for snake bite is lack of neutralization of all toxic component of snake venom as venom is composed of numerous enzymes of various size and nature. Procoagulant and lethal effects of Pakistan Russell viper venom moderately neutralized by the Indian VINS polyvalent Antivenom (VPAV). On a concentration dependent manner, VPAV immunologically bind to few of the venom protein and immunorecognition was weak to moderate for PLA A2 and other small proteins (Faisal et al., 2018). In a similar study the enzymatic and pharmacological effects of southern India Russell's viper was neutralized by polyvalent Antivenom (PAV). However, PAV fail to neutralize the PLA A2 and venom induced indirect hemolytic activity, due to poor recognition of low molecular weight of protein (Kalita et al., 2018). Results of these studies may be correlated with the chronic complications or inability to regain normal life in humans underwent immunotherapy.

4. Conclusion

A.paniculata a promising medicinal plant is on spot light getting explored for various biological activities including venom neutralization. Result of the present study clearly points out the possibility of *A. paniculata* inneutralizing venom by targeting PLA A2, a potent neurotoxin and myotoxin exist in most of the snake venom. Deoxyandrographolide and Andrograpanin present in *A. paniculata* are found to have a strong interaction with PLA A2 at LYS69 and GLY33 (catalytic site) studied in AutoDock 4.0. The available immunotherapy failed to neutralize PLA A2 because of its small size. Hence, the small molecules either from natural source or synthetic can be used as an adjuvant (in addition to immunotherapy) to completely neutralize snake venom in order to address the complication associated with snake envenom thereby reducing the mortality rate. The results highly recommend further biochemical studies on the phytochemicals of *A. paniculata* against a various component of snake venom especially PLA A2.

Acknowledgment

Authors thank B. S. Abdur Rahman Crescent Institute of Science and Technology for providing research facility.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101058.

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