In vitro and *in silico* validation of anti-cobra venom activity and identification of lead molecules in *Aegle marmelos* (L.) Correa

N. C. Nisha[1], S. Sreekumar[1,*], D. A. Evans[2] and C. K. Biju[1]

[1]Saraswathy Thangavelu Centre, Biotechnology and Bioinformatics Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Puthenthope, Thiruvananthapuram 695 586, India
[2]Department of Zoology, University College, Thiruvananthapuram 695 034, India

Venomous snakebite is a global serious health issue and in India high rate of mortality is caused by *Naja naja* (Indian cobra). To evaluate anti-cobra venom activity and identify lead molecules in *Aegle marmelos*, *in vitro* and *in silico* screening was carried out. Leaves, stem and root bark of *A. marmelos* were extracted in ethanol, methanol and hexane and maximum yield was obtained in methanol. All extracts were used for testing *in vitro* anti-haemolytic, inhibition of anti-acetylcholinesterase and anti-proteolytic activities. The results revealed that ethanol extract of root bark has high anti-haemolytic activity, methanol extracts of leaves have the highest inhibitory effect on venom induced anti-acetylcholinesterase activity and ethanol extracts of leaves have maximum anti-proteolytic activity. Docking between 81 phytochemicals from *A. marmelos* and each of the 14 cobra venom toxic proteins revealed that the plant contains potential molecules for detoxification of all the cobra venom proteins.

Keywords: *Aegle marmelos*, cobra venom, docking, phytochemicals, snakebite.

THE annual global snakebite death rate is ~125,000, of which 50,000 are Indians[1,2] and 80% of the victims depend on traditional healers and such details are not properly documented. Considering the high death rate, the World Health Organisation has included snakebite in the list of neglected tropical diseases. Snake venom is a complex mixture of bioactive compounds such as enzymatic and non enzymatic proteins, peptides, lipids, nucleotides, carbohydrates and amines. Of these, 90% of dry weight constitutes proteins[3]. Over 62 pharmacologically active[4] and 20–25 toxic molecules have been reported from snake venom, of which 12 toxic proteins are common, which may induce cytotoxicity, neurotoxicity, haemotoxicity, cardiotoxicity and myotoxicity[5]. The venom composition may vary from species to species, habitat and age of the snake. Due to this complexity and inconsistency, it is difficult to identify a single medicine against snakebite. Immunotherapy is the only treatment against snake envenomation in modern medicine. However, it induces serious side effects such as anaphylaxis, inflammation and immune reaction in patients. The scarcity of quality venom, its storage, inconsistency in venom composition, high cost, etc. are other constraints in immunotherapy. Since time immemorial, herbal medicines have been used against snakebites. In India over 350 plant species have been reported as antidotes to snake venom, but their efficacy and molecular mode of drug action have seldom been scientifically demonstrated.

Among the four common venomous snakes in India, a high death rate is caused by the Indian cobra (*Naja naja*)[6]. *Aegle marmelos* is a common medicinal tree distributed throughout India with its root, stem bark and leaves being used against snake envenomation[7,8]. However, there is a controversy on its anti-snake venom activity[9]. In these backdrops, the present study was aimed to evaluate the anti-cobra venom activity of *A. marmelos* through *in vitro* method, and identify lead molecules against each cobra venom toxic protein through *in silico* method.

Materials and methods

In vitro anti-cobra venom activity assays

Preparation of plant extracts and venom sample: Leaves, stem and root bark of *Aegle marmelos* collected from 5 to 10-year-old field grown plants were shade dried and powdered separately. Ten gram powdered samples of each plant part was soaked separately in 100 ml hexane, ethanol and methanol for 72 h. The extracts were filtered using Whatman no. 1 filter paper and concentrated. Each extract was dried and total yield was estimated. The dried extracts were dissolved in normal saline after mixing with Tween20 and used for further experiments. A herbarium specimen of the experimental plant was deposited in the JNTBGRI herbarium with accession number TBGT30702. Lyophilized venom of *Naja naja* was procured from Haffkine Institute, Parel, Mumbai, and preserved at 4°C (No. HITRT/ZNS-VAU/VM/43/1047).

*For correspondence. (e-mail: drsreekumar@rediffmail.com)

Table 1. Experimental set up for determining anti-haemolytic activity of plant extract against cobra venom

Cell suspension	Test materials added	Observation
RBCs 60 µl	Distilled water 660 µl	100% haemolysis
	PBS 660 µl	Control
	PBS 600 µl + 60 µl cobra venom	Hemolysis
	PBS 600 µl + 60 µl cobra venom + 60 µl plant extract	Inhibition of haemolysis

Anti-haemolytic activity: Anti-haemolytic activity was determined following the method of Vijayabharathi *et al.*[10]. Around 5 ml blood from a healthy human volunteer was collected using a sterile syringe with the help of a nurse and immediately poured into Alsevier's solution[11]. The sample was centrifuged at 2000 rpm for 10 min and the supernatant was poured out. The pellet containing RBCs was washed twice with physiological saline and a 20% suspension in phosphate buffered saline (PBS) was used as the substrate for hemolysis. Table 1 shows the experimental set up for determining anti-haemolytic activity of plant extract against cobra venom.

The samples were kept in a thermostat water bath at 37°C for 1 h, and then centrifuged at 2000 rpm for 20 min. The optical density of the supernatant fluid was measured using a spectrophotometer at a wavelength of 540 nm against water.

Percentage of hemolysis = (OD of experimental sample/ OD of control) × 100.

Inhibition of cobra venom induced anti-acetylcholinesterase activity: Acetylcholinesterase (Ach-E) activity was estimated by the method of Augustinson[12]. The fresh brain tissue of domestic fowl was dissected out and 300 mg tissue was homogenized at ice cold condition in 3 ml phosphate buffer and centrifuged at 2000 rpm for 10 min. The supernatant was used as enzyme source. Table 2 shows the experimental set up for determining inhibition of cobra venom induced anti-Ach-E activity by the extract of *A. marmelos.*

All the samples were incubated at 37°C for 30 min. Two ml alkaline hydroxyl amine solution was then added to each sample with thorough mixing. Subsequently, 1 ml HCl solution and 1 ml $FeCl_3$ solution were added. The absorbance was measured at 540 nm using distilled water as blank.

Activity of Ach-E was expressed as the amount of acetylcholine degraded by the enzyme/mg protein. Amount of acetylcholine was quantified by running standard solution of acetylcholine with the experiment.

% of Ach-E activity = (Concentration of standard/ OD of standard) × OD of experimental sample.

Specific activity (Amount of acetylcholine liberated per hour per mg protein) = (Activity of experimental sample/ Amount of protein in enzyme source) × OD of experimental sample.

Anti-proteolytic activity: Cathepsin D was assayed done as described by Mycek[13]. It was based on the appearance of free amino acid which was released during the digestive process. The components can be measured in the acid filtrate by the production of a blue coloured product with Folin–Ciocalteu reagent. The liver tissue of domestic fowl (200 mg) was homogenized using ice cold citrate buffer. The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was taken as the enzyme source. Bovine haemoglobin (2.5%) was taken as substrate. The experimental set up for determining anti-proteolytic activity is shown in Table 3.

The samples were incubated at 38°C for 10 min and the reaction was stopped by adding 2 ml of 5% trichloroacetic acid. The samples were thoroughly mixed, allowed to stand at room temperature for 10 min and filtered using Whatman no. 3 filter paper. Small aliquots from the filtrate were subjected to protein estimation with Folin–Ciocalteau reagent and optical density of each sample was measured using a spectrophotometer at a wavelength of 660 nm.

Under *in vitro* condition the plant extracts effectively inhibited the proteolytic activity induced by cobra venom.

Unit of activity (in 7 ml) = OD of experimental sample × 70

% of viability = (Unit of activity/Amount of protein in enzyme source) × 100.

In silico screening

Preparation of macromolecules: As reported earlier[14,15], fourteen cobra (*Naja naja* L.) venom toxic proteins, viz. phospholipase A2 (PLA2), long neurotoxin 1 (LN1), long neurotoxin 2 (LN2), long neurotoxin 3 (LN3), long neurotoxin 4 (LN4), long neurotoxin 5 (LN5), acetylcholinesterase (Ach-E), L-aminoacid oxidase (L-AAO), cobramin A (CA), cobramin B (CB), cytotoxin 3 (CYT3), cobrotoxin (COT), serine protease (SP) and proteolase (PL) were prepared as the receptor molecules for docking. The active sites of all protein molecules were detected using the tools Q-site finder and Pocket finder.

Table 2. Experimental set up for determining inhibition of cobra venom induced anti-acetylcholinesterase activity by the extract of *Aegle marmelos*

Test materials added	Observation
Buffered substrate 940 µl + buffer 160 µl	Blank
Buffered substrate 940 µl + enzyme 60 µl + buffer 100 µl	Normal activity 100% activity
Buffered substrate 940 µl + enzyme 60 µl + venom 50 µl + buffer 50 µl	Inhibition of enzyme by venom
Buffered substrate 940 µl + enzyme 60 µl + venom 50 µl + plant extract 50 µl	Prevention of anti-acetylcholinesterase activity induced by cobra venom

Table 3. Experimental set up for determining anti-proteolytic activity

Test materials added	Observation
Haemoglobin substrate 1 ml + enzyme 250 µl + dist. water 250 µl + buffer 250 µl	100% proteolysis
Haemoglobin substrate 1 ml + enzyme 250 µl + dist. water 250 µl + buffer 200 µl + cobra venom 50 µl	Elevation in proteolytic activity
Haemoglobin substrate 1 ml + enzyme 250 µl + dist. water 250 µl + buffer 150 µl + cobra venom 50 µl + plant extract 50 µl	Prevention of venom induced proteolytic activity by plant extract

Table 4. Anti-haemolytic, inhibition of venom induced anti-acetylcholinesterase and anti-proteolytic activities of different parts of *Aegle marmelos* extracts in various organic solvents

		Inhibition of anti-acetylcholinesterase activity		Anti-proteolytic activity	
Plant extract 1 mg	Anti-haemolytic activity (%)	Acetylcholinesterase activity (%)	Specific activity (µg)	Proteolytic activity (%)	Specific activity (IU)
Control	Nil	100	568.54	100	5.09
Venom	20**	27.00**	480.75^{++}	133$^{\#\#}$	7.39$^{\#\#}$
Leaf + ethanol	5.71	23.60	421.35	120	6.69$^{\#}$
Leaf + methanol	11.42	27.12	482.9^{+}	133	7.39
Leaf + hexane	11.42	23.90	427.12	133	7.39
Stem + ethanol	5.71	9.88	176.04	133	7.39
Stem + methanol	25.71*	19.79	352.09	49.85	2.77
Stem + hexane	6.21	17.00	303.03	133	7.39
Root + ethanol	31.45*	22.00	392.49	133	7.39
Root + methanol	11.42	11.34	202.02	87.28	4.85
Root + hexane	7.32	12.80	227.99	133	7.39

**Cobra venom induced haemolysis considered as 100% haemolysis – venom concentration 25 µg/assay medium.
*Anti-haemolytic activity by plant extract.
$^{++}$Cobra venom 25 µg/assay mixture caused 27% inhibition of acetylcholinesterase activity – considered as 100% inhibition.
$^{+}$Indicate highly effective antagonistic activity by plant extract.
$^{\#\#}$Cobra venom 25 µg/assay mixture caused 33% elevation of cathepsin D activity.
$^{\#}$The plant extracts which brought the activity to control value is considered as positive effect.

Preparation of ligands: Out of 81 phytochemicals reported from *A. marmelos*, the canonical simplified molecular input line entry system (SMILES) of 61 compounds was retrieved from PubChem; the remaining 20 molecules were drawn (Figure 1) using ACD/ChemSketch and 3D structures of all molecules were generated using CORINA.

Docking: All selected phytochemicals were docked into the binding site of each of the fourteen cobra venom proteins using AutoDock 4.2 following the procedure described by Morris *et al.*[16]. The active site prediction, grid spacing, selection of all parameters for docking and docked results analysis were done as reported earlier[14,17]. The ligand-protein bound complexes were analysed for their binding affinity and possible orientations were ranked according to their lowest binding energy through cluster analysis. The top-ranked molecules with free energy of binding ≤ –5 kcal/mol were considered as hit molecules.

Drug-likeliness prediction using molinspiration tool: To analyse the drug-likeliness properties, the hit molecules were submitted on the molinspiration property prediction tool[18] and analysed as reported earlier[17].

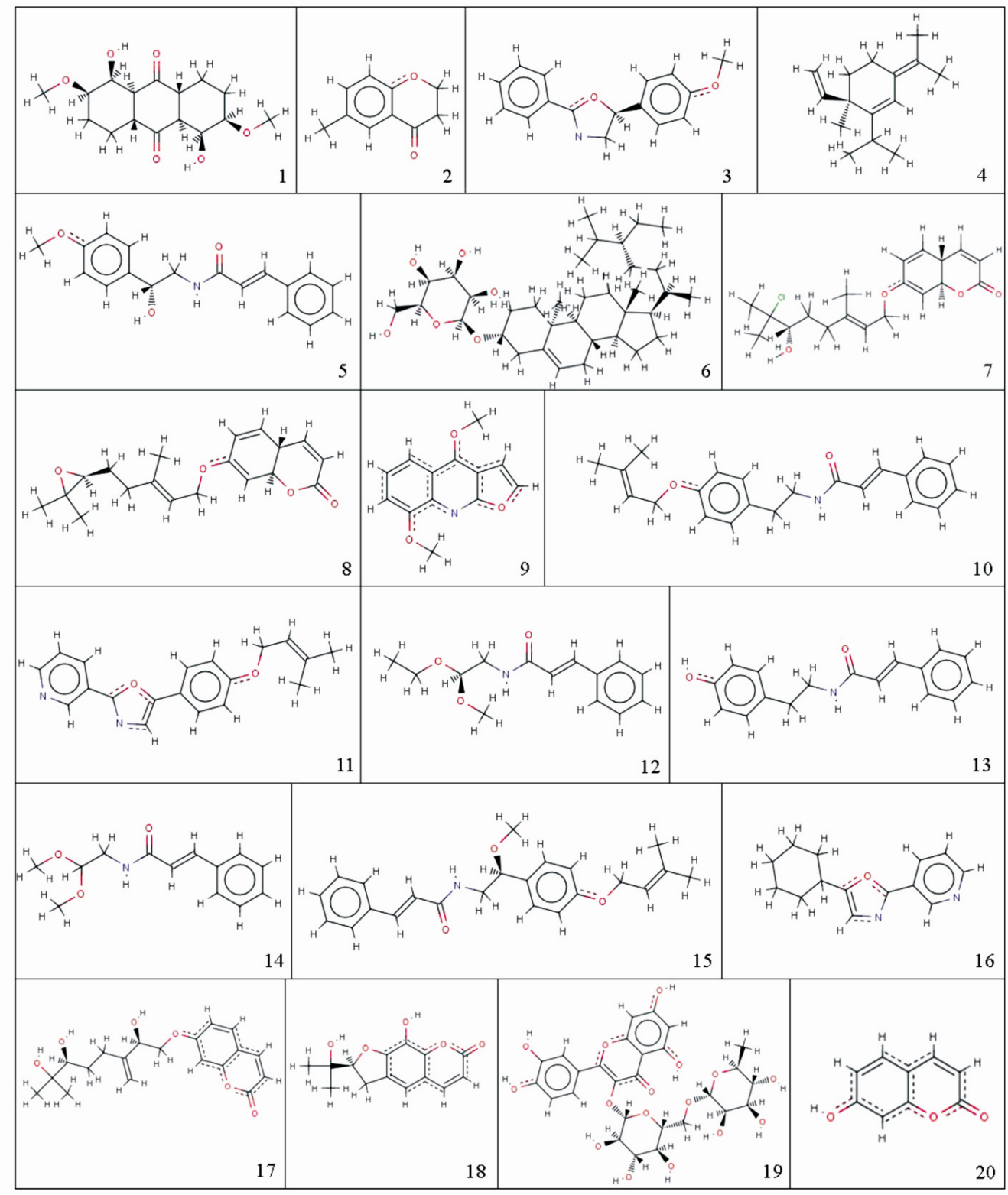

Figure 1. Structure of phytochemicals created: 1, 1-5-Dihydroxy-6-methoxy-2-methylanthraquinone; 2, 6-Methyl-4-chromanone; 3, Aeglemarmelosine; 4, α-Elemene; 5, β-Phenyl ethyl amides aegeline; 6, β-Sitosterol β-D-glucoside; 7, Chloromarmin; 8, Epoxyauraptene; 9, Fagarine; 10, Marmeline; 11, Montaine; 12, N-2-hydroxy-2-[4-hydroxy phenyl) ethyl cinnamide; 13, N-2-ethoxy-2-(4-methoxy phenyl) ethyl cinnamide; 14, N-2-methoxy-2-(4-methoxy phenyl) ethyl cinnamide; 15, N-2-methoxy-2-[4(3′,3′-dimethylallyloxy phenyl)] ethyl cinnamide; 16, O-isopentenyl halfordinol; 17, Praealtin D; 18, Rutaretin; 19, Rutin; 20, Umbelliferone.

Table 5. Selected lead molecules from *Aegle marmelos* against 14 cobra venom proteins

Lead molecules	Venom proteins	ΔG_{bind} (kcal/mol)	Inhibition constant (KI)	H-bond	Bond type	Bond length (Å)
Chloromarmin	PLA2	–9.23	172.56 nM	TYR63:HH	O–H..O	1.86
Epoxyauraptene		–9.2	180.92 µM	GLY29:HN 1	N–H..O	2.098
				TYR63:HH 1	O–H..O	1.935
Aurapten		–8.7	420.77 nM	GLY29:HN 1	O–H..O	1.95
				TYR63:HH 1	N–H..O	1.91
Decursinol		–8.41	685.03 nM	HIS47:HD1 1	O–H..O	1.98
				TYR63:HH1	O–H..O	1.99
Marmeline		–8.29	840.16 nM	TYR27:H43 1	N–H..O	1.99
				HIS47:HD1 1	N–H..O	1.87
Marmin		–8.25	900.69 nM	TYR27:H48	O–H..O	1.83
Aeglemarmelosine		–8.08	1.2 µM	No H-bonds		
O-isopentenyl halfordinol		–8.03	1.29 µM	HIS47:HD1 1	N–H..N	2.05
Chloromarmin	COT	–9.63	89.05 nM	No H-bonds		
Anhydromarmeline		–9.58	95.38 µM	No H-bonds		
Marmin		–9.26	164.16 nM	No H-bonds		
Marmeline		–9.12	207.5 nM	No H-bonds		
Lupeol		–9.04	236.59 nM	No H-bonds		
Psoralen (ficusin)	LN1	–5.03	205.12 µM	THR22:O5 1	O–H..O	2.37
Psoralen (ficusin)	LN2	–5.38	114.68 µM	THR22:O12	O–H..O	2.49
				PRO64:O5	O–H..O	2.864
Linalool		–5.06	194.75 µM	PRO64:H26	O–H..O	1.93
				THR22:HG1	O–H..O	2.1
Linalool	LN3	–5.10	183.13 µM	PRO71:H26	O–H..O	1.931
				GLN55:HE21	N–H..O	1.92
Psoralen (ficusin)	LN4	–5.04	203.14 µM	THR22:O5 1	O–H..O	2.428
Halfordinol (Aegelinine)		–5	224.94 µM	VAL37:HN 1	N–H..O	1.92
Psoralen (ficusin)	LN5	–5.94	44.52 µM	No H-bonds		
Myrtenyl acetate		–5.64	73.3 µM	No H-bonds		
Halfordinol (Aegelinine)		–5.33	124.92 µM	VAL37:HN 1	N–H..O	2
Linalool		–5.2	153.07 µM	GLN55:HE21	N–H..O	2.2
β-Phellandrene	CA	–5.01	225.43 µM	No H-bonds		
Psoralen	CB	–5	220.14 µM	No H-bonds		
α-Pinene	CYT3	–5.58	66.14 µM	No H-bonds		
β-Sitosterol	PL	–7.28	4.6 µM	CYS304:H67	N–H..O	1.732
				ARG269:HH22	O–H..O	1.762
Marmesinin (Nodakenin)		–7.26	4.78 µM	ASN278:H52	O–H..O	1.84
				ASN279:H50	O–H..O	1.54
				ASN305:HD21	N–H..O	1.98
Lupeol		–7.22	5.12 µM	No H-bonds		
Chloromarmin	SP	–5.7	65.89 µM	ALA16:HN 1	N–H..O	2.06
				TRP17:HN 1	N–H..O	2.07
				LYS34:HZ2 1	N–H..O	2.06
Halfordinol (Aegelinine)		–5.66	71.55 µM	THR27:H28 1	N–H..O	1.86
				LYS28:HN 1	O–H..O	1.76
Epoxyaurapte-ne		–5.35	119.58 µM	LYS34:HZ2 1	N–H..O	1.73
Aeglemarmel-osine		–5.08	189.85 µM	No H-bonds		
β-Sitosterol		–5.04	202.34 µM	No H-bonds		
α-Amyrin	L-AAO	–10.01	46.21 nM	No H-bonds		
Lupeol		–9.3	153.68 µM	TYR389:HH	O–H..O	2.23
β-Sitosterol		–9.09	216.69 nM	SER445:H67 1	O–H..O	1.91
γ-Sitosterol		–8.98	263.16 nM	ARG90:H67 1	O–H..O	2.08
γ-Sitosterol	AchE	–7.52	3.09 µM	TYR63:H67	O–H..O	2.17
β-Sitosterol		–7.52	3.06 µM	TYR63:H67	O–H..O	2.1

Results and discussion

In vitro anti-venom activity studies

Snake venom consists of different types of innumerable biologically active molecules and its individual constituents, quality and quantity are unstable and unpredictable. Similarly, the plant extract also contains a plethora of chemical constituents which are formulated through biological processes and its quality and quantity may not always be stable. However, the synergistic effect of chemical constituents in a plant extract can successfully

Table 6. Prediction of drug-likeliness properties of lead molecules in *Aegle marmelos* using the tool molinspiration

Lead molecule	miLogP	TPSA	Atoms	MW	#ON	#OHNH	#Violations	#ROTB	Volume
Chloromarmin	3.5	55.77	24	352.9	4	1	0	7	326.2
Myrtenyl acetate	3	26.3	14	194.3	2	0	0	3	196.6
Psoralen	2.29	43.4	14	186.2	3	0	0	0	154.2
Linalool	3.21	20.23	11	154.3	1	1	0	4	175.6
Halfordinol	2.51	59.2	18	238.3	4	1	0	2	208.13
Cineol	2.72	9.23	11	154.3	1	0	0	0	166.66
Valencic acid	3.58	46.53	15	206.2	3	1	0	4	197.37
α-Pinene	3.54	0	10	136.2	0	0	0	0	151.81
Marmesinin	0.47	138.8	29	408.4	9	4	0	4	350.12
α-Amyrin	8.08	20.23	31	426.7	1	1	1	0	461.05
β-Sitosterol	8.62	20.23	30	414.7	1	1	1	6	456.52
γ-Sitosterol	8.62	20.23	30	414.7	1	1	1	6	456.52

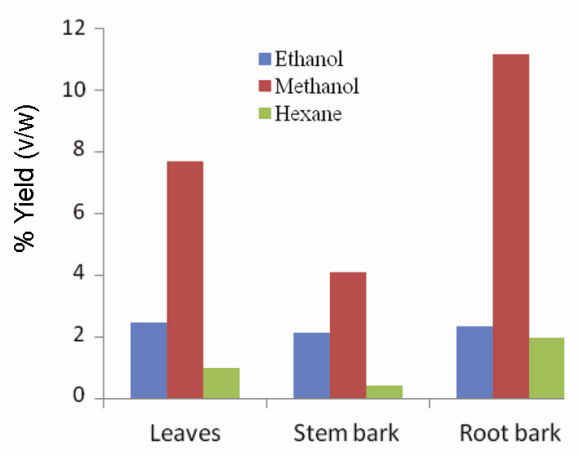

Figure 2. Different parts of *Aegle marmelos* percentage yield in various solvants.

neutralize multi-toxicity or pathogenesis inducing factors simultaneously. Therefore, as reported by earlier workers[19–22], various plant parts such as stem bark, root bark and leaves were extracted in ethanol, methanol and hexane. The percentage yield of the plant extracts depended on the solvent and the plant part used. Among the three solvents used, methanolic extracts showed high yield (Figure 2). Generally, for extracting bio-molecules from plants, methanol is preferred due to its amphiphilic nature and low boiling point.

In cobra venom lethality is mainly caused by cardiotoxins and neurotoxins. The lysis of erythrocytes by cardiotoxins is a ready and reproducible test and it was observed in 85% of the snake venom samples tested[23]. PLA2 is a major cardiotoxin in cobra venom. The haemolytic assay results revealed that all the tested plant extracts have anti-haemolytic activity at various levels (Table 4). The minimum concentration of plant extract for optimum anti-haemolytic activity was 1 mg. The ethanolic extracts of root bark have high inhibitory activity and the methanolic extracts of stem bark have moderate inhibitory activity.

Inhibition of cobra venom induced anti-acetylcholinesterase activity

The cobra venom inhibits the activity of Ach-E and this inhibition was prevented by plant extracts at various levels (Table 4). The leaf methanolic extract of *A. marmelos* showed the highest inhibitory effect (77.7%) on venom induced anti-Ach-E activity. Negligible low quantities of Ach-E enzyme present in cobra venom may not affect the neurotransmission of the host or victim but Ach-blockers or inhibitors can directly act on the synapse of the neurone–neurone junction and neuron–muscle junction. The effectiveness of plant extracts on the activity of Ach-blockers or inhibitors in cobra venom has significant effect on the victim. Mostly, instantaneous death due to cobra bite is through inhibition of muscle contraction of the intercostal muscles in association with ribs and chest, thereby preventing respiration.

Anti-proteolytic activity

Cobra venom induced 33% elevation in cathepsin D activity. The ethanolic extracts of the leaves of *A. marmelos* under *in vitro* condition have successfully inhibited the elevated proteolytic activity by cobra venom (Table 4) and the result was in corroboration with an earlier study that herbal drugs have potential inhibitory effect on venom induced proteolysis[24].

Docking

The docked results between 81 phytochemicals from *A. marmelos* and each of the 14 venom proteins revealed that the plant contains potential molecules for detoxification of all cobra venom proteins. Most molecules showed moderate inhibitory activity (≤–5 kcal/mol) on six venom proteins. In the order of merit, L-AAO ranked first with 75 hit molecules followed by COT (73), PLA2 (66), CB (59), Ach-E(4), proteolase (3) and CYT3 (24). Several lead molecules have pleotropic venom protein inhibitory

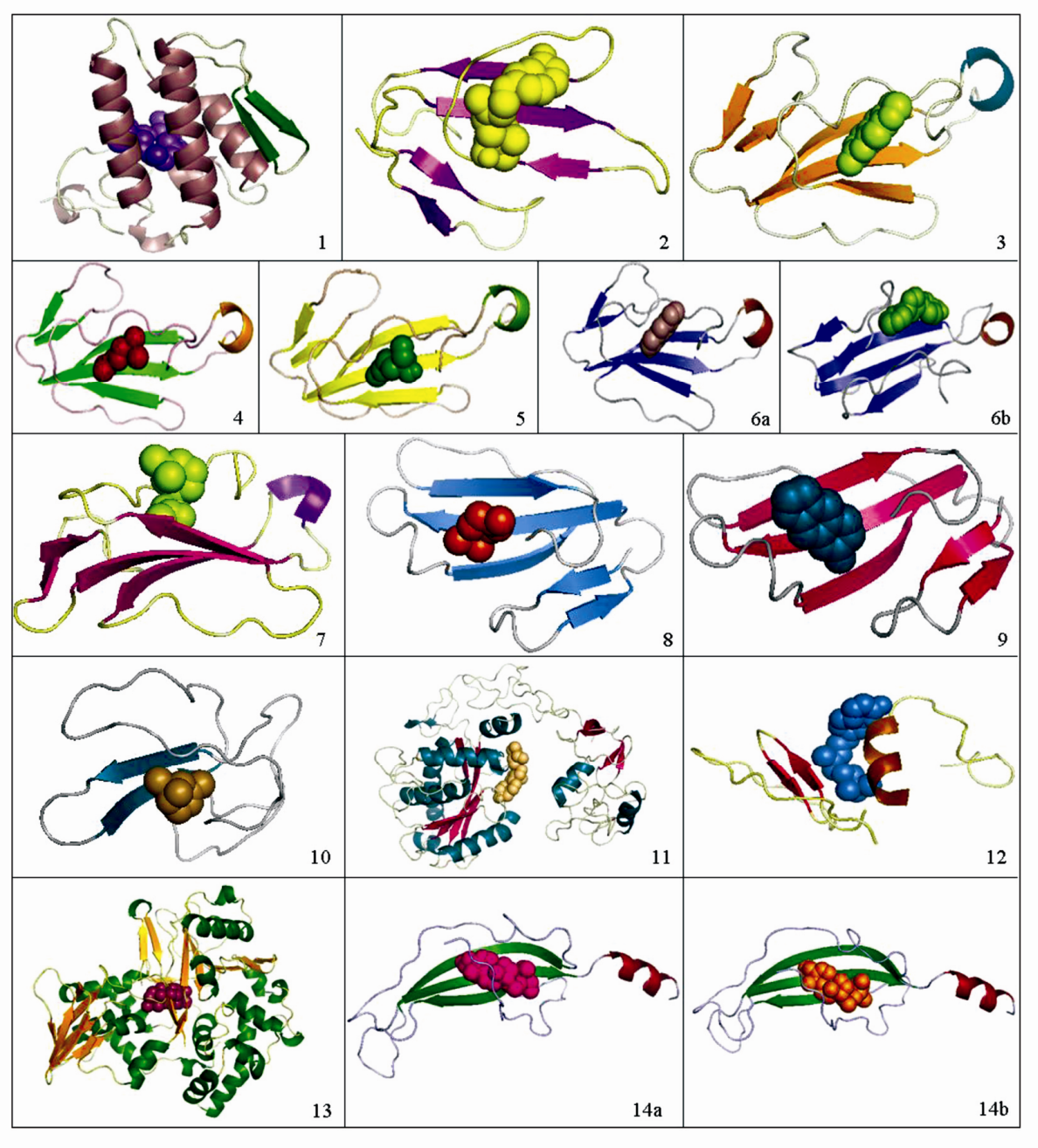

Figure 3. Venom proteins and lead molecules: 1, PLA2 and chloromarmin; 2, COT and Chloromarmin; 3, LN1 and Psoralen; 4, LN2 and Linalool; 5, LN3 and Linalool; 6a, LN4 and Halfordinol; 6b, LN4 and Psoralen; 7, LN5 and Psoralen; 8, CA and β-phellandrene; 9, CB and Psoralen; 10, CYT3 and α-pinene; 11, PL and β-sitosterol; 12, SP and Chloromarmin; 13, L-AAO and α-amyrin; 14a, Ach-E and β-sitosterol; 14b, Ach-E and γ-sitosterol.

activity. The lead molecules from this plant showed lowest ΔG_{bind} on proteins such as PLA2, COT, CB, CYT3, L-AAO and Ach-E. The molecular interaction of the lead molecules and the proteins showed that except with COT and LN5, in all other cases, H-bonds were formed between the ligand and residue at the active site of proteins. The bond lengths were from 1.7 Å to 2.2 Å and bond types were N–H..O and O–H..O (Table 5). Of the drug-likeness prediction of the selected twelve molecules, three of them, viz. β-amyrin, β-sitosterol and γ-sitosterol violate miLogP value (Table 6). The 3D views of the docked structures are depicted in Figure 3. The docked results confirmed that *A. marmelos* is a potential antidote plant to treat snakebite especially against *N. naja* bite.

1. Mohapatra, B., Warrell, D. A., Suraweera, W., Bhatia, P. and Dhingra, N., Snakebite mortality in India: A nationally representative mortality survey. *PLoS. Negl. Trop. Dis*., 2011, **5**, e1018.

2. Kasturiratne, A. *et al.*, The global burden of snakebite: A literature analysis and modelling based on regional estimates of envenoming and deaths. *PLoS Med*., 2008, **5**, e218.
3. Lalla, J. K., Sunita, O., Priyanka, G., Zaid, T. and Geeta T., Snakebite problem in India: An overview. *Sch. Acad. J. Pharm.*, 2013, **2**, 252–259.
4. Nisha, N. C., Sreekumar, S., Biju, C. K. and Krishnan, P. N., Snake antivenom: Virtual screening of plant derived molecules. *Biobytes*, 2010, **6**, 14–22.
5. Vyas, V. K., Brahmbhatt, K., Bhatt, H. and Parmar, U., Therapeutic potential of snake venom in cancer therapy: current perspectives. *Asian Pac. J. Trop. Biomed*., 2013, **3**, 156–162.
6. Binh, D. V., Thanh, T. T. and Chi, P. V., Proteomic characterization of the thermostable toxins from *Naja naja* venom. *J. Venom. Anim. Toxins Incl. Trop. Dis*., 2010, **16**, 631–638.
7. Singh, K. K., Kalakoti, B. S. and Prakash A., Traditional phytotherapy in the healthcare of Gond tribals of Sonbhadra district, UttarPradesh, India. *J. Bombay Nat. Hist. Soc*., 1994, **91**, 385–390.
8. Raju, M. S., Native plants used in snakebite and other poisonous animals among the tribals of East Godavari district, Andhra Pradesh. *Aryavaidyan*, 1996, **9**, 251–255.
9. Kirtikar, K. R. and Basu, B. D., Indian medicinal plants. In second edition (eds Balatter, E., Caius, J. F. and Mhaskar, K. S), Bishen Singh Mahendra Pal Singh, Dehradun, India, 2004, pp. 499–502.
10. Vijayabharathi, R. A., Kumar, S. and Kumar, K. S., *In vitro* and *in vivo* anti-snake venom activity of *Coccinia indica* L. leaf. *Hamdard Med*., 2005, **48**, 132–135.
11. Alsever, J. B. and Ainslie, R. B., A new method for the preparation of dilute blood plasma and the operation of a complete transfusion service. *N. Y. State J. Med*., 1941, **41**, 126–131.
12. Augustinson, Assay methods for cholinesterase. In *Methods of Biochemical analysis* (ed. Glick, D.), Wiley, New York, 1957, p. 43.
13. Mycek, M. J., Cathepsin methods. *Enzymology*, 1970, **9**, 285–315.
14. Nisha, N. C., Sreekumar, S., Biju, C. K. and Krishnan, P. N., Identification of lead compounds with cobra venom neutralizing activity in three Indian medicinal plants. *Int. J. Pharm. Pharm. Sci.,* 2014, **6**, 536–541.
15. Peitsch, M. C., Protein modeling by e-mail Bio/Technology. *Nat. Biotechnol*., 1995, **13**, 658–660.
16. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J., AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.*, 2009, **30**, 2785–2791.
17. Nisha, N. C., Sreekumar, S. and Biju, C. K., Identification of lead compounds with cobra venom detoxification activity in *Andrographis paniculata* (Burm. F.) Nees through *in silico* method. *Int. J. Pharm. Pharm. Sci.*, 2016, **8**, 212–217.
18. http://www.molinspiration.com
19. Mitul, P., Handral, M. and Swaroop, T. V. S. S., Anti-venom activity of *Cinnamomum zeylanicum* extracts against *Naja kauthia* snake venom. *Int. J. Pharm. Chem. Biol. Sci*., 2013, **2**, 1302–1310.
20. Maiorano, V. A. *et al.*, Antiophidian properties of the aqueous extract of *Mikania glomerata*. *J. Ethnopharmacol*., 2005, **102**, 364–370.
21. Alam, M. I. and Gomes, A., Snake venom neutralization by Indian medicinal plants (*Vitex negundo* and *Emblica officinalis*) root extracts. *J. Ethnopharmacol*., 2003, **86**, 75–80.
22. Monimala, M. and Mukherjee, A. K., Neutralisation of lethality, myotoxicity and toxic enzymes of *Naja kaouthia* venom by *Mimosa pudica* root extracts. *J. Ethnopharmacol*., 2001, **75**, 55–60.
23. Soto, J. G., Perez, J. C. and Minton, S. A., Proteolytic, hemorrhagic and hemolytic activities of snake venoms. *Toxicon*, 1988, **26**, 875–882.
24. Janardhan, B., Shrikanth, V. M., Mirajkar, K. K. and More, S. S., *In vitro* screening and evaluation of antivenom phytochemicals from *Azima tetracantha* Lam. leaves against *Bungarus caeruleus* and *Vipera russelli*. *J. Venom. Anim. Toxins Incl. Trop. Dis.*, 2014, **1**, 1.

ACKNOWLEDGEMENTS. We thank INSPIRE Programme, Department of Science and Technology, Government of India, New Delhi for financial support. We also thank the Director, JNTBGRI, The Principal, University College, Thiruvananthapuram and Dr T. Madhan Mohan, Advisor, DBT for providing the facilities and encouragement.

Received 26 June 2017; revised accepted 12 December 2017

doi: 10.18520/cs/v114/i06/1214-1221