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Validation of Russell's Viper Venom Detoxification Activity of *Azadirachta indica* through *In Silico* Method

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Abstract : *Azadirachta indica A. Juss. (neem) is one of the Indian medicinal plants used as antidote to snake venom particularly against viper envenomation. In the present study 335 phytochemicals reported from Azadirachta indica were docked with Daboia russelii venom proteins, basic phospholipase A_2 VRV-PL-VIIIa (PDB id 1OXL) and class II anticoagulant phospholipase A_2 (PDB id 1VIP) using the tool AutoDock 4.2. To avoid error in lead identification, the top ranked hit molecules (ΔGbind<-10 kcal/mol) were docked using different docking tools such as SwissDock, PatchDock, iGemDock and Hex. The results were analysed following Dempster Shafer Theory (DST) and also the docked structures were analysed in Ligplot$^+$. The compound 1-acetyl-7-tigloyl-nimbidinin and 1-senecioyl-3-acetyl vilasinin lactol were identified as the leads against phospholipase A_2 VRV-PL-VIIIa and nimbocinolide, 1-cinnamoyl vilasinin lactone and tirucallol were identified as leads against class II anticoagulant phospholipase A_2.*

Keywords : *AutoDock, Daboia russelii, Phospholipase A_2, Venom, Viper , Azadirachta indica, Neem*

I. Introduction

The mortality and morbidity due to snake bite is a major health problem particularly among the rural population in tropical countries like India. Annually, 5.0-5.5 million people are bitten by snakes resulting 40,000 amputations and 20,000 - 1,25,000 deaths in all over the world. In India annual snake bite death rate is 15,000 to 50,000[1] and most of the death caused by the bites of the "Big Four" venomous snakes *viz*. *Naja naja*, *Bungarus caeruleus*, *Daboia russelii* and *Echis carinatus* [2]. Russell's viper (*Daboia russelii*) causes high morbidity and mortality in Southern India. It is widely distributed throughout East Asia, Southeast and South Asia. *Daboia russelii russelii* (Indian Russell's viper) is the common species in India and Pakistan. It has a well-developed dentition and venom apparatus suitable for inflicting a deadly bite [3].

Venom of Russell's viper can induce neurotoxicity, myotoxicity, haemolysis, coagulopathy, renal failure, severe necrosis and hypopituitarism [4,5]. It contains many toxic enzymatic proteins *viz.* serine proteinases, Zn^{2+}-metalloproteinases, L-amino acid oxidase, phospholipase A_2 (PLA_2) and non-enzymatic toxic proteins such as disintegrins, C-type lectins, CRISP (Cysteine Rich Secretary Proteins), nerve and vascular endothelium growth factors etc. Of these, PLA_2 is the major enzyme (upto 70%) which may present in the form of at least seven isoenzymes [6] induces high toxicity and lethality. Immunotherapy is the only treatment against snake envenomation in modern medicine which induces serious side effects such as serum sickness reactions, anaphylactic shock and pyrogen reaction. Geographical variation in venom composition, identification of snake species and non-availability of storage facility in rural areas are the major obstacles in anti-venom therapy and these all well discussed [7]. Most of the snake bite victims (>90%) depend on herbal medicines [8] which contain a plethora of chemical molecules that are synthesized within the living system. Therefore, the chance of side effects from plant derived molecules may be negligible and they can neutralize the toxicity of several venom proteins at a time. Formulation of a drug against a multifactorial causation like snake venom in modern medicine is a herculean task. A combination of drug molecules acting simultaneously against a number of targets is likely to be more effective than drug acting at one target, which may be possible only in herbal medicine.

Globally about 600 plant species and in India about 350 plant species have been reported as anti-snake venom plants. However, its efficacy and molecular mechanism of drug action are seldom investigated. Identification of potential lead molecule against the target molecule is the first step in drug discovery and conventionally it is achieved through high throughput screening which is rather time consuming and expensive. While *in silico* screening is more direct and rational drug discovery approach which is less expensive, quick, effective and screening can be done without the low volume high value plant derived molecules.

Azadirachta indica is a well known Indian medicinal plant which has been used as a polychrest to treat a variety of ailments in traditional medicine and its medicinal properties got global acceptance [9]. One of the major uses of this plant is against snake venom particularly to treat Russell's viper envenomation [10,11,12]. About 335 secondary compounds were reported from this plant belong to the group triterpenoids (isoprenoids),

flavonoids and coumarins (non-isoprenoids) [13,14,15,16,17,18,19]. The present investigation was aimed to the *in silico* screening and identification of potential lead molecules against the toxic venom proteins *viz*. basic phospholipase A_2 VRV-PL-VIIIa and class II anticoagulant phospholipase A_2 (RVV-VD), the major toxins present in Russell's viper venom which cause lethality in human.

II. Materials and Methods

1.1. Preparation of the receptor molecules

The 3D structures of selected target proteins, basic PLA_2 VRV PL VIIIa (PDB id-1OXL) and class II anticoagulant phospholipase A_2 (RVV-VD) (PDB id- 1VIP) isolated from the venom of Russell's viper were downloaded from RCSB Protein Data Bank. The first protein (1OXL) consists of 121 amino acid residues with crystallographically independent A and B chains which form the asymmetric homodimer. It has an R value of 1.8 A^0. The protein contains a natural ligand identified as an indole derivative, 2-carbamoyl-methyl-5-propyl-octahydro-indol-7-yl-acetic acid (IDA). The ligand was bound to the substrate binding site in the chain A and involved in the interaction with the catalytic residues His48 and Asp49 and it was absent in chain B. The second target protein (PDB id- 1VIP) consists of a single chain with 121 amino acid residues. Prior to docking, the natural ligands and the water molecules present in the proteins were removed using SwissPdbViewer. Identification of pocket sites on the protein surface is often the starting point for structure-based drug design and a prerequisite for protein–ligand docking. Active site slot of the secretary PLA_2s was found to be ~15 A^0 deep and present within the catalytic residues. N-terminal region of PLA_2 contains interfacial binding site with specific affinity for lipid-water interfaces. Identification of active site of the target proteins was done using MetaPocket2. It is a consensus method, which combines the predictions done by LIGSITECS, PASS, Q-SiteFinder, SURFNET, Fpocket, GHECOM, ConCavity and POCASA [20]. Both target proteins in PDB format were uploaded on the server and the output showed the predicted binding pockets of the proteins. Among the residue molecules in the binding site, Asp49 was selected as the active site residue for the docking.

1.2. Preparation of the ligand molecules

Perusal of literatures and search on open access chemical databases, a list of 335 chemical molecules (TABLE 1) derived from *Azadirachta indica* A. Juss. (Meliaceae) were selected as ligand molecules. Of these, the structures of 182 compounds were retrieved from "neem" metabolite structure database (http://www.vmsrf.org/trial_neem/index.php?db=1), 130 from PubChem database (http://pubchem.ncbi.nlm.nih.gov/), three from ChemSpider and rest of them from various literatures[13,14,15,16,17,18,19]. Chemical structures of fifteen molecules were not available on open access databases and literatures and those structures were drawn and generated SMILES notations using ChemSketch. The 3D structures of all compounds were created using the tool CORINA.

1.3. Docking

For preliminary screening, docking was carried out using the open access application tool, AutoDock 4.2 following the standard procedure [21]. AutoDock uses a semi empirical force field to predict binding free energies of small molecules to macromolecules. The tool uses Monte Carlo Simulated Annealing and Lamarckian Genetic Algorithm for the possible orientation of ligands at the binding site of each protein. The numbers of grid points in xyz co-ordinates were set as 70×70×40 respectively with a spacing of 0.375 A^0. All docking parameters were kept as default. AutoDock runs several times to get various docked conformations. It generated best ten poses and they were scored using scoring functions [22]. Best docked complexes were analysed and ranked according to the lowest possible free energy of binding. Molecules with lowest free energy of binding less than $^-$10kcal/mol were selected as hit molecules.

1.4. Post docking analysis

Dempster Shafer Theory (DST) was used for the rank sum analysis of the docked results. The docked results obtained using above mentioned various tools were documented in an excel spread sheet (.xls file format) and uploaded on the web tool (http://allamapparao.org/dst/). DST uses a five steps procedure for the analysis. They are: (1) class generation (dividing data into four classes), (2) get result from Rank Sum technique, (3) get result from DST unweighted, (4) get result from DST weighted, (5) get result from Zhang rule. Top ranked molecules from second to fifth were selected as true hits and proposed as lead molecules for further evaluation. The docked structures were also analysed in Ligplot$^+$ and determined the structural details and mode of interactions.

III. Results and Discussion

Snake venom phospholipase A_2(s) the major component of all venomous snakes particularly in viper venom cause neurotoxicity, myotoxicity, cardiotoxicity, and hemorrhagic and anticoagulant effects. It

hydrolyses the cellular or sub cellular membrane phospholipids and generates products which are lytic and cause membrane damage [23,24,25]. Secreted PLA_2s are characterized by a highly conserved Ca^{2+} binding loop, a catalytic site with a His-Asp dyad and six conserved disulfide bonds [26]. The enzyme specifically catalyze the hydrolysis of the acyl ester bond at the sn-2 position of 1,2-diacyl-3-sn-phoshoglycerides and promote the release of lysophospholipids and fatty acids *viz* arachidonic acid. Arachidonic acid is the precursor of eicosanoids such as prostaglandins, thromboxanes, leukotrienes and platelet activating factors. These eicosanoids cause inflammatory disorders such as vasodilation, vasoconstriction, increased vascular permeability, inhibition or promotion of platelet aggregation, hyperalgesic etc. Excess level of PLA_2 also associated with physiopathological effects such as cardiovascular disorders, cancers, asthma, cerebral diseases etc. [27,28,29]. Hence inhibition of secretary PLA_2 leads to the decrease of eicosanoids levels and such a way inflammatory problems can be controlled.

Protein structure analysis of the PDB structure of basic phospholipase A_2 VRV-PL-VIIIa revealed that, the target protein was under hydrolase class, and its secondary structure was incorporated with 47% helices and 8% beta sheets. The helical structure consists of 7 helices with 58 residues where as the beta sheet consists of 4 strands with 10 residues. The MetaPocket2 server output consists of a binding site with 82 amino acid residues which were distributed in both chain A and B. Active site of the protein contains both the catalytic dyad residues, His48 and Asp49 [30]. For the present study, Asp49 was selected as the active site residue. Even though His48 is an important residue in snake venom PLA_2s, calcium binding activity was performed in the presence of Asp49. Asp49 bind with Ca ions first and the interaction was favoured by the negative charge of Asp49 [31]. Thus it controls the Ca binding activity and became an essential residue for the hydrolysis of phospholipids [32]. Replacing Asp49 with any other residues at 49^{th} position was also showed changes in enzyme activity [33]. At physiological pH, aspartic acid gets deprotonated and interacts with the polar environment, thus maintain the solubility and ionic characters of the protein.

Docked conformations were predicted based on the free energy of binding of ligands to the receptor molecules. The compound showing lesser free energy of binding has higher inhibitory activity [34]. Generally, molecules showed free energy of binding $\leq$ -5.00 kcal/mol were considered as hit molecules but here majority of the compounds having free energy of binding less than the former level and therefore molecules having free energy $\leq$ -11.00 kcal/mol were considered as the hit molecules. Thus, a total of 13 compounds were identified as hits against the basic phospholipase A_2 VRV-PL-VIIIa (TABLE 2).

Among these, 1-acetyl-7-tigloylnimbidinin showed least free energy of binding and two hydrogen bonds, one at the ALA18 residue with a bond distance of 2.158 Å and the other at the GLY 30 residue with a bond distance of 1.989 Å. Among the other hits identified, 1-senecioyl-3-acetylvilasininlactol showed two H-bonds with Asp49 with a bond distance of 2.233 Å and GLY30 with a bond distance of 1.828 Å. Sitosterol showed one H-bond interaction with ALA18 residue with a bond length of 2.216 Å. Rest of the hits not showed any H-bond interaction. Details of H-bond interactions were given in TABLE 3. The compound 1-acetyl-7-tigloylnimbidinin showed least binding energy but it had no H-bond with the catalytic residue Asp49. The top hit molecules identified were again docked using SwissDock, PatchDock, iGEMDOCK and Hex Server. Top ranked molecules were further subjected to consensus scoring to improve the scoring reliability and hit rate in virtual screening. Consensus scoring combines information from various docked scores of the same problem to reduce errors in single scores and improve the probability of identifying true ligands [35]. The docked results were statistically analyzed following DST method and selected the best lead molecule TABLE 4. Among the 13 molecules, 1-acetyl-7-tigloylnimbidinin was the top ranked molecule in AutoDock and DST analysis and considered as one of the lead molecules. It is a limonoid coming under the phytochemical class tetranortriterpenoid and present in neem seeds. The enzymatic activity of phospholipase A_2 is absolutely dependent on the presence of calcium ions and it was well demonstrated that the residue Asp49 controls calcium binding [36]. 1-senecioyl-3-acetyl vilasinin lactol showed H- bond interaction with the Asp49 residue of the target protein and therefore it was also suggested as a best lead molecule against basic phospholipase A_2 VRV-PL-VIIIa. It is a limonoid coming under the phytochemical class tetranortriterpenoid and present in fresh leaves of neem (Fig.1). Several pentacyclic triterpenes derived from plants also shown antiphospholipasic A_2 activity [37, 38].

Protein structure analysis of the second target, RVV-VD revealed that, it belongs to the class hydrolase and its secondary structure consists of 48% helices and 9% beta sheets. The helical structure consists of 7 helices with 59 residues where as the beta sheets composed of 5 strands with 11 residues. The MetaPocket2 server output showed a binding site containing 72 amino acid residues which were distributed in a single chain. The docked results in AutoDock showed that out of 335 phytochemicals screened sixteen of them showed high binding affinity and free energy of binding less than -10.00 kcal/mol and these molecules were selected as hit molecules. They were nimbocinolide (-14.44 kcal/mol), stigmasterol (-13.28 kcal/mol), 2,3-dehydro salanol (-12.52 kcal/mol), azadirachtin F (-12.07 kcal/mol), stigmast-4-en-3-one (-10.67 kcal/mol), cycloeucalenone (-10.63 kcal/mol), 1-cinnamoyl vilasinin lactone (-10.54 kcal/mol), limocinin (-10.54 kcal/mol), nimolinone (-10.46 kcal/mol), nimbocinol (-10.45 kcal/mol), epoxy-azadiradione benzoate (-10.31 kcal/mol), 7alpha-

senecioyl-7-deacetyl-23-o-methylnimocinolide (˜10.27kcal/mol), kulactone (˜10.16 kcal/mol), nimbosterol (˜10.12 kcal/mol), tirucallol (˜10.08 kcal/mol) and 7-deacetyl 7-benzoylnimbinin (˜10.06 kcal/mol). Details of hit molecules were shown in TABLE 5. Among the 16 molecules, nimbocinolide showed least free energy of binding. It is a terpenoid present in neem leaves. 1-cinnamoyl vilasinin lactone showed H-bond interaction with GLU53 (bond distance of 2.40 Å), nimbocinolide showed one hydrogen bond with PRO18 residue (bond distance of 2.024 Å), 2, 3-dehydrosalanol with HIS48 (bond distance of 1.932 Å), azadirachtin F showed two H-bond interactions, one with TYR22 (bond distance of 2.059 Å) and other with GLY30 (bond distance of 1.989 Å), nimbocinol showed H-bond interactions with CYS45 (bond distance of 1.94 Å) and HIS48 (bond distance of 2.160 Å), epoxy-azadiradione benzoate with GLY30 (bond distance of 2.881Å), nimbosterol with SER23 (bond distance of 2.008 Å), tirucallol with ASP49 (bond distance of 2.233 Å), and 7-deacety 7- benzoyl nimbinin with GLY30 (bond distance of 2.061 Å). Details of H-bond interactions were given in the TABLE 3. Hit molecules were again docked with other docking tools followed by DST analysis and the results were depicted in TABLE 4. The molecule 1-cinnamoyl vilasinin lactone was a top ranked molecule in DST analysis. It had not shown bonding with Asp49 in AutoDock but the Ligplot result clearly showed bonding with Asp49 (Fig 2). It is a γ-hydroxybutenolide under the class tetranortriterpenoid which was reported from neem leaves. Similarly, in AutoDock outputs showed that the compound tirucallol had H-bond interaction with Asp49 residue but the same interaction had not clearly observed in Ligplot output. The molecule Nimbocinolide showed least free energy of binding in AutoDock but H bond interaction with Asp49 was not observed. However, the Ligplot output indicated that the other interactive binding forces were comparatively high and bonding was observed with one of the active residues His48. It was also reported that the consensus scoring method like DST is not always gave accurate results. In these backdrops, the molecules 1-cinnamoyl vilasinin lactone, tirucallol and nimbocinolide were identified as best leads against the target protein RVV-VD and recommended for further evaluation.

IV. Conclusion

The overall results indicated that the plant contains potential phytochemicals for detoxification of snake venom proteins particularly against viper venom. The results strongly substantiated the antidote property of neem, however, *in vitro* and *in vivo* experimental demonstration is essential.

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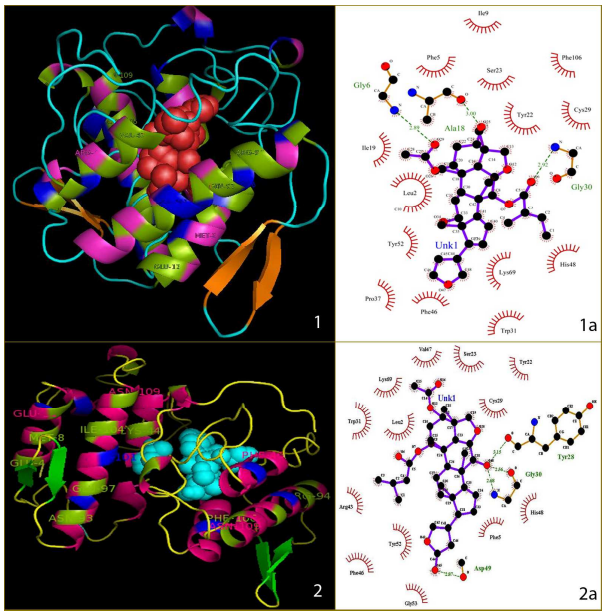

Figure 1: Docked pose and H-bond interactions of lead molecules with basic phospholipase A_2VRV-PL-VIIIa in AutoDock (1) 1-acetyl-7-tigloylnimbidinin (1a) Interactions represented in LigPlot$^+$ (2) 1-senecioyl-3-acetyl vilasinin lactol (2a) Interactions represented in LigPlot$^+$

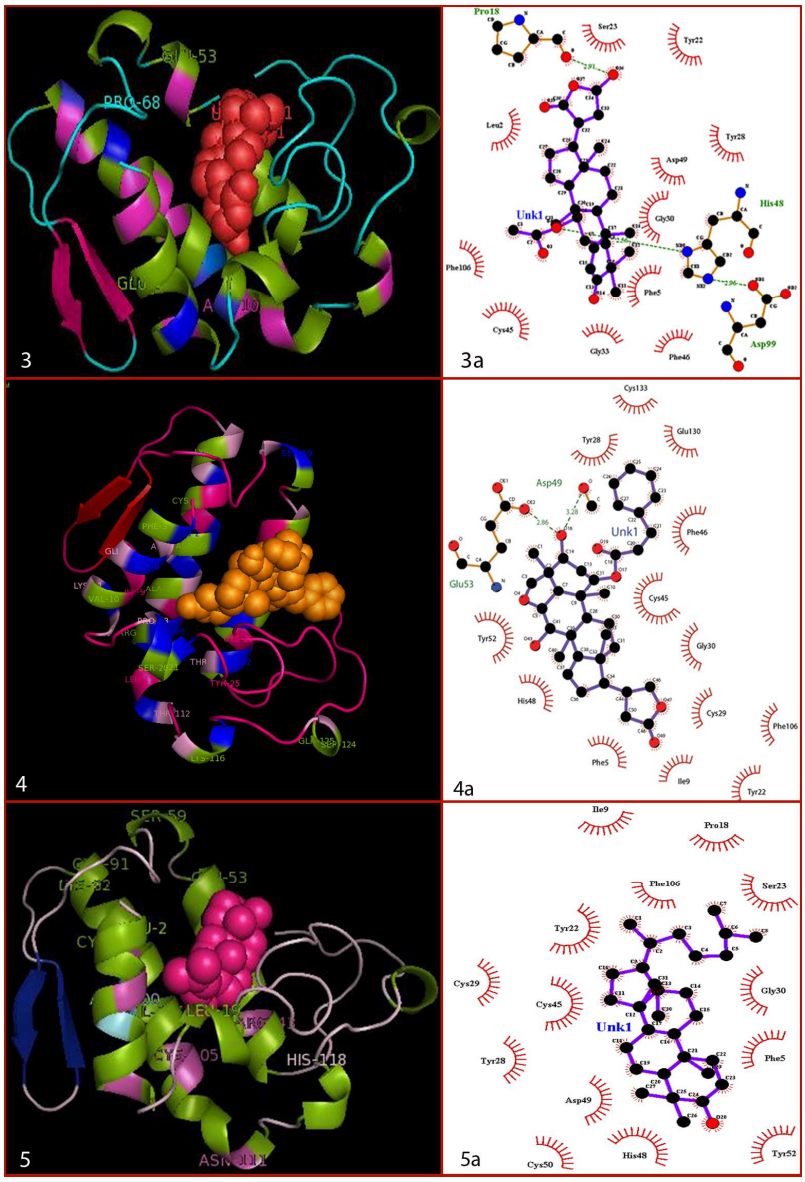

Figure 2: Docked pose and H-bond interactions of lead molecules with class II anticoagulant phospholipase A_2 in AutoDock: (3) Nimbocinolide; (3a) Interactions represented in LigPlot$^+$; (4) 1-cinnamoylvilasininlactone; (4a) Interactions represented in LigPlot$^+$; (5) Tirucallol; (5a) Interactions represented in LigPlot$^+$

TABLE 1: List of selected Phytochemicals from *Azadirachta indica* for docking

Sl.No	Compound; Molecular Formula; Molecular Weight	Sl.No	Compound; Molecular Formula; Molecular Weight
1.	(-)-Epicatechin; $C_{15}H_{14}O_6$; 290.26806	169	Isoazadiradionolide; $C_{28}H_{36}O_6$; 468.5818
2.	(+)-Germacrene A; $C_{15}H_{24}$; 204.35106	170	Isoazadirolide; $C_{32}H_{42}O_{10}$; 586.6699
3.	1,2-Dihydro-4-alpha,6-alpha-dihydroxy-A-homozadirone; $C_{29}H_{38}O_6$; 482.60842	171	Isoazadironolide; $C_{28}H_{38}O_6$; 470.5977
4.	1,3-Diacetyl-11, 19-deoxa-11-oxo-meliacarpin; $C_{31}H_{40}O_{12}$; 604.6421	172	Isofraxidin; $C_{11}H_{10}O_5$; 222.1941
5.	1,3-Diacetyl-12-alpha-acetoxyvilasinin; $C_{32}H_{42}O_9$; 570.6705	173	Isomargolonone; $C_{19}H_{22}O_4$; 314.375
6.	1,3-Di-o-Acetylvilasinin; $C_{30}H_{40}O_7$; 512.6344	174	Isomargosinolide; $C_{27}H_{32}O_8$; 484.5382
7.	11-Demethoxycarbonyl-11-oxomeliacarpin; $C_{32}H_{40}O_{12}$; 616.6528	175	Isomeldenin; $C_{28}H_{38}O_5$; 454.5983
8.	11-Epi-azadirachtin D; $C_{34}H_{44}O_{14}$; 676.27310612	176	Isonimbinolide; $C_{30}H_{36}O_{11}$; 572.6002

9.	1-3-Diacetyl-7-tigloyl-12-hydroxyvilasinin; $C_{35}H_{46}O_9$; 610.7343	177	Isonimbocinolide; $C_{32}H_{44}O_{10}$; 588.6858
10.	1-3-Diacetylvilasinin; $C_{29}H_{40}O_6$; 484.6243;	178	Isonimbolide; $C_{30}H_{38}O_9$; 542.6173
11.	14,15,20,21-Diepoxy-23-nimonolactone; $C_{29}H_{38}O_8$; 514.60722	179	Isonimocinolide; $C_{28}H_{36}O_7$; 484.58124
12.	14,15-Epoxy-16-beta-hydroxyazadirone ; $C_{28}H_{36}O_6$; 468.58184	180	Isonimolicinolide; $C_{30}H_{40}O_9$; 544.6332
13.	14-15-Deoxygedunin; $C_{28}H_{34}O_7$; 482.5654	181	Isonimolide; $C_{29}H_{40}O_7$; 500.6237
14.	14-15-Epoxynimonol; $C_{28}H_{36}O_5$; 452.5824	182	Iso-Scopoletin; $C_{10}H_8O_4$; 192.16812
15.	17-Alpha-hydroxyazadiradione; $C_{28}H_{34}O_6$; 466.566	183	Isovepaol; $C_{36}H_{48}O_{17}$; 752.7561
16.	17-Beta-Hydroxyazadiradione; $C_{28}H_{34}O_6$; 466.566	184	Kaempferol 3-o-glucoside; $C_{21}H_{20}O_{11}$; 448.3769
17.	17-Beta-hydroxynimbocinol; $C_{26}H_{32}O_5$; 424.5293	185	Kaempferol; $C_{15}H_{10}O_6$; 286.2363
18.	17-Epi-azadiradione; $C_{28}H_{34}O_5$; 450.5666	186	Kaempferol3-o-beta-D-glucoside; $C_{21}H_{20}O_{11}$; 448.3769
19.	17-Epi-nimbocinol ; $C_{26}H_{32}O_6$; 408.5299	187	Kaempferol-3-o-rutinoside; $C_{27}H_{30}O_{15}$ C; 594.5181
20.	1-Acetyl-7-tigloylnimbidinin ; $C_{33}H_{42}O_8$; 566.6818	188	Khivorin; $C_{31}H_{40}O_{11}$; 588.6427
21.	1-Acetyl-7-tigloylvilasinin; $C_{33}H_{44}O_7$; 552.6983	189	Kulactone; $C_{30}H_{44}O_3$; 452.66856
22.	1-Alpha-2alpha-epoxynimbinin; $C_{27}H_{34}O_6$; 454.5553	190	Kulinone; $C_{30}H_{48}O_2$; 440.70092
23.	1-Alpha-methoxy-1,2-dihydroazadiradione; $C_{30}H_{40}O_7$; 512.6344	191	Lanosterol; $C_{30}H_{50}O$; 426.7174
24.	1-Alpha-methoxy-1-2-dihydronimbinin; $C_{29}H_{38}O_7$; 498.6078	192	Lignocericacid; $C_{24}H_{48}O_2$; 368.6367
25.	1-Beta-2 beta-epoxynimbinin; $C_{28}H_{34}O_7$; 482.5654	193	Limbocidin; $C_{33}H_{40}O_{13}$; 644.6629
26.	1-Cinnamoylvilasininlactone; $C_{35}H_{44}O_7$; 576.7197	194	Limbocinin; $C_{32}H_{38}O_{12}$; 614.6369
27.	1-Detigloyl-1-isobutyroyl-epoxymethacroylazadirachtin; $C_{34}H_{44}O_{16}$;708.7036	195	Limbonin; $C_{35}H_{48}O_{13}$; 676.7478
28.	1-Detigloyl-1-isocaproyl-3-deacetyl-3-epoxymethacroylazadirachtin; $C_{38}H_{50}O_{17}$; 778.7934	196	LimocinA; $C_{31}H_{44}O_6$; 512.6775
29.	1-Detigloyl-1-isovaleroylazadirachtin; $C_{34}H_{46}O_{15}$; 694.72	197	LimocinB; $C_{29}H_{42}O_5$; 470.6408
30.	1-O-tigloyl-3-o-acetylvilasinin; $C_{30}H_{41}O_6$; 497.6423914	198	Limocin-C; $C_{30}H_{44}O_5$; 484.6674
31.	1-Senecioyl-3-acetylvilasinin; $C_{33}H_{44}O_7$; 552.6983	199	Limocin-D; $C_{30}H_{44}O_5$; 484.6674
32.	1-Senecioyl-3-acetylvilasininlactol; $C_{33}H_{48}O_8$; 572.7294	200	Limocinin; $C_{32}H_{42}O_5$; 506.6729
33.	1-Senecioyl-3-acetylvilasininlactone; $C_{33}H_{46}O_8$; 570.7135	201	Limocinol; $C_{28}H_{46}O$; 398.66424
34.	1-Tigloyl-3-acetyl-11-methoxyazadirachtinin; $C_{38}H_{50}O_{16}$; 762.794	202	Limocinone; $C_{28}H_{44}O$; 396.6484
35.	1-Tigloyl-3-acetylvilasinin; $C_{33}H_{44}O_7$; 552.6983	203	Limonene; $C_{10}H_{16}$; 136.23404
36.	1-Tigloyl-3-acetylvilasininlactol; $C_{33}H_{46}O_8$; 570.7135	204	Limonin; $C_{26}H_{30}O_8$; 470.5116
37.	1-Tigloyl-3-deacetatyl-12-alpha-acetoxyvilasinin; $C_{37}H_{48}O_9$; 636.77162	205	Longifolene; $C_{15}H_{24}$; 204.35106
38.	1-Tiglyoyl-3-acetyl-12a-acetoxyvilasinin; $C_{34}H_{44}O_{10}$; 612.7072	206	Mahmoodin; $C_{30}H_{38}O_8$; 526.61792
39.	2, 4-Heptadienal; $C_7H_{10}O$; 110.15	207	Margocetin; $C_{12}H_{14}O_4$; 222.2372
40.	2,3-Dihydronimbicacid; $C_{26}H_{32}O_8$; 472.5275	208	Margocilin1; $C_{20}H_{28}O_3$; 316.4345
41.	21-Oxo-ohchinolide ; $C_{35}H_{44}O_{11}$; 640.7173	209	Margocilin2; $C_{20}H_{28}O_3$; 316.4345
42.	22-23-Dihydroazadirachtin; $C_{30}H_{40}O_{15}$; 640.6296	210	Margocin; $C_{20}H_{26}O_2$; 298.4192

43.	22-23-Dihydronimocinol; $C_{28}H_{40}O_5$; 456.6142	211	Margocinin; $C_{20}H_{26}O_4$; 330.418
44.	2-3-Dehydro-salannol; $C_{32}H_{42}O_8$; 554.6711	212	Margolone; $C_{19}H_{24}O_3$; 300.39206
45.	23-Desmethyllimocin; C28H40O5; 456.6142	213	Margolonone; $C_{19}H_{22}O_4$; 314.37558
46.	24-Methylenecycloartanol; $C_{31}H_{52}O$; 440.74398	214	Margosinolide; $C_{27}H_{32}O_8$; 484.5382
47.	24-Methylenecycloartanone; $C_{31}H_{50}O$; 438.7281	215	Margosinone; $C_{29}H_{50}O_4$; 462.7049
48.	28-Deoxonimbolide; $C_{27}H_{32}O_6$; 452.5394	216	Margosolone; $C_{18}H_{24}O_3$; 288.3814
49.	2-Pinen-4-one; $C_{10}H_{14}O$; 150.21756	217	Margosone; $C_{21}H_{30}O_3$; 330.4611
50.	2-Prime-3prime-dihydrotigloyl-22, 23-dihydroazadirachtol; $C_{33}H_{44}O_{14}$; 664.6941	218	Meldenin; $C_{28}H_{36}O_5$; 452.5824
51.	2-Prime-3prime-dihydrotigloylazadirachtol; $C_{33}H_{44}O_{14}$; 664.6941	219	Meldenindiol; $C_{26}H_{34}O_4$; 410.5458
52.	2-Undecanone; $C_{11}H_{22}O$; 170.29178	220	Meliacinanhydride; $C_{30}H_{38}O_8$; 526.6179
53.	3-Acetyl-7-tiglyolvilasininlactone; $C_{33}H_{46}O_8$; 570.7135	221	Meliacinin; $C_{31}H_{44}O_6$; 512.6775
54.	3-Deacetyl-3-cinnamoylazadirachtin; $C_{42}H_{48}O_{16}$; 808.8209	222	Meliacinol; $C_{31}H_{40}O_8$; 540.6445
55.	3-Deacetylazadirachtin; $C_{33}H_{42}O_{15}$; 678.6776	223	Meliacinolactol; $C_{36}H_{46}O_7$; 590.7462
56.	3-Deacetylsalannin; $C_{32}H_{42}O_8$; 554.6711	224	Melianin; $C_{41}H_{58}O_9$; 694.89382
57.	3-Desacetylazadirachtolide; $C_{31}H_{48}O_7$; 532.7086	225	Melianodiol; $C_{30}H_{48}O_5$; 488.6991
58.	3-Isobutyroylazadirachtol; $C_{32}H_{42}O_{14}$; 650.6675	226	Melianol; $C_{30}H_{48}O_4$; 472.6997
59.	3-O-Deacetyl-azadirachtin; $C_{33}H_{44}O_{15;}$ 680.6935	227	Melianone; $C_{30}H_{46}O_4$; 470.68384
60.	3'-Prenylnaringenin; $C_{20}H_{18}O_{5;}$ 338.35392	228	Meliantriol; $C_{30}H_{50}O_5$; 490.715
61.	3-Tigloyl-22-23-dihydroazadirachtol; $C_{33}H_{44}O_{14}$; 664.6941	229	Meliaquinal; $C_{31}H_{40}O_{13}$; 620.6415
62.	4a-Hydroxy-A-homo-isomeldenin; $C_{28}H_{38}O_6$; 470.266839	230	Meliatetraolenone; $C_{34}H_{48}O_9$; 600.7395
63.	4-Alpha-6-alpha-dihydroxy-alpha-homoazadirone; $C_{28}H_{36}O_6$; 468.5818	231	Meliatetraone; $C_{29}H_{36}O_7$; 496.5919
64.	4-Epinimbin; $C_{30}H_{36}O_{9;}$ 540.6014	232	Methyl 8-(2-furyl)octanoate; $C_{13}H_{20}O_3$; 224.2961
65.	6-Acetyl-nimbandiol; $C_{28}H_{34}O_8$; 498.225368	233	Methyl(2E,6E)-farnesoate; $C_{16}H_{26}O_2$; 250.37644
66.	6a-O-Acetyl-7-deacetylnimocinol; $C_{28}H_{36}O_5$; 452.5824	234	Methylnimbiol; $C_{21}H_{30}O_2$; 314.4617
67.	6-Deacetyl-isonimbinolide; $C_{28}H_{34}O_{10}$; 530.5636	235	Myricetin; $C_{15}H_{10}O_8$; 318.2351
68.	6-Deacetylnimbin; $C_{26}H_{32}O_6$; 440.5287	236	Myristicacid; $C_{14}H_{28}O_2$; 228.3709
69.	6-Deacetylnimbinal; $C_{27}H_{32}O_7$; 468.5388	237	Naheedin; $C_{32}H_{48}O_6$; 528.71992
70.	6-Deacetylnimbinene; $C_{26}H_{32}O_{6;}$ 440.5287	238	Nakanishi; $C_{37}H_{48}O_{15}$; 732.76802
71.	6-Deacetylnimbinolide; $C_{29}H_{36}O_{8;}$ 512.5913	239	Neeflone; $C_{28}H_{38}O_4$; 438.5989
72.	6-Deacetylphotonimbin; $C_{29}H_{36}O_{10;}$ 544.5901	240	Nimbaflavone; $C_{26}H_{30}O_5$; 422.5134
73.	6-Hydroxymellein; $C_{10}H_{10}O_4$; 194.184	241	Nimbanal; $C_{29}H_{34}O_8$; 510.5755
74.	6-Methoxymellein; $C_{11}H_{12}O_4$; 208.21058	242	Nimbandiol; $C_{26}H_{32}O_7$; 456.5281
75.	7-Acetylneotrichilenone ; $C_{28}H_{36}O_5$; 452.5824	243	Nimbicacid; $C_{26}H_{30}O_8$; 470.5116
76.	7-Alpha-senecioyl-7-deacetyl-23-o-methylnimocinolide ; $C_{32}H_{40}O_8$; 552.6552	244	Nimbidicacid; $C_{28}H_{38}O_8$; 502.5965
77.	7-Benzoylnimbocinol; $C_{32}H_{36}O_4$; 484.6258	245	Nimbidin; $C_{26}H_{34}O_6$; 442.54456
78.	7-Deacetoxy-7-hydroxygedunin ;	246	Nimbidinin; $C_{26}H_{34}O_6$; 442.54456

	$C_{26}H_{32}O_6$; 440.52868		
79.	7-Deacetyl-7-benzoylgedunin; $C_{32}H_{36}O_6$; 516.6246	247	Nimbidiol; $C_{17}H_{22}O_3$; 274.3548
80.	7-Deacetyl-7-benzoylnimbinin; $C_{32}H_{36}O_6$; 516.6246	248	Nimbilicin; $C_{20}H_{24}O_3$; 312.4028
81.	7-Deacetylazadirone; $C_{26}H_{34}O_3$; 394.54636	249	Nimbilin; $C_{42}H_{50}O_{10}$; 714.8404
82.	7-Deacetylgedunin; $C_{26}H_{32}O_6$; 440.5287	250	Nimbin; $C_{30}H_{36}O_9$; 540.60144
83.	7-Detigloyl-7-methacroyl-11-deacetylnimbolidinA; $C_{36}H_{48}O_{11}$; 656.7597	251	Nimbinene; $C_{28}H_{34}O_7$; 482.5654
84.	7-Detigloyl-7-senecioyl-11-deacetylnimbolidin-A; $C_{36}H_{48}O_{11}$; 656.319662	252	Nimbinicacid; $C_{27}H_{32}O_8$; 484.5382
85.	7-Tigloyl-12alpha-acetoxyvilasinin; $C_{34}H_{44}O_8$; 580.70836	253	Nimbinol; $C_{27}H_{34}O_7$; 470.5547
86.	Acetylmeliacinolactol; $C_{45}H_{58}O_{13}$; 806.387742	254	Nimbinolide; $C_{30}H_{36}O_{11}$; 572.6002
87.	Alpha pinene; $C_{10}H_{16}$; 136.23404	255	Nimbinone; $C_{18}H_{22}O_3$; 286.36548
88.	Alpha-cubebene; $C_{15}H_{24}$; 204.35106	256	Nimbiol; $C_{18}H_{24}O_2$; 272.38196
89.	Alpha-dihydrogedunol; $C_{28}H_{38}O_7$; 486.261754	257	Nimbione; $C_{18}H_{22}O_3$; 286.36548
90.	Alpha-himachalene; $C_{15}H_{24}$; 204.35106	258	Nimbionol; $C_{18}H_{24}O_4$; 304.3808
91.	Ascorbic acid; $C_6H_8O_6$; 176.12412	259	Nimbionone; $C_{18}H_{22}O_4$; 302.3649
92.	Azadirachtanin A; $C_{32}H_{40}O_{11}$; 600.257062	260	Nimbisonol; $C_{18}H_{24}O_3$; 288.3814
93.	Azadirachtanin; $C_{30}H_{10}O_4$; 542.61732	261	Nimbocetin; $C_{24}H_{38}O_4$; 390.5561
94.	Azadirachtin A; $C_{35}H_{44}O_{16}$; 720.71426	262	Nimbochalcin; $C_{22}H_{26}O_9$; 434.4364
95.	Azadirachtin B; $C_{35}H_{44}O_{16}$; 720.71426	263	Nimbocidin1; $C_{20}H_{30}O_2$; 302.451
96.	Azadirachtin D; $C_{34}H_{44}O_{14}$; 676.70476	264	Nimbocidin2; $C_{20}H_{26}O_4$; 330.418
97.	Azadirachtin E; $C_{33}H_{44}O_{15}$; 680.69346	265	Nimbocin; $C_{40}H_{54}O_8$; 662.852
98.	Azadirachtin F; $C_{32}H_{44}O_{12}$; 620.6846	266	Nimbocinin; $C_{19}H_{26}O_4$; 318.4073
99.	Azadirachtin G; $C_{33}H_{42}O_{15}$; 678.252371	267	Nimbocinol; $C_{26}H_{32}O_4$; 408.5299
100.	Azadirachtin H; $C_{33}H_{42}O_{14}$; 662.67818	268	Nimbocinolide; $C_{28}H_{36}O_6$; 468.5818
101.	Azadirachtin I; $C_{32}H_{42}O_{12}$; 618.6687	269	Nimbocinone; $C_{30}H_{46}O_4$; 470.68384
102.	Azadirachtin K; $C_{34}H_{40}O_{15}$; 688.6724	270	Nimbolicin; $C_{41}H_{48}O_{10}$; 700.8138
103.	Azadirachtin L; $C_{35}H_{44}O_{15}$;7 04.71486	271	Nimbolicinol; $C_{33}H_{46}O_{10}$; 602.7123
104.	Azadirachtin M; $C_{35}H_{44}O_{15}$; 704.71486	272	Nimbolide; $C_{27}H_{30}O_7$; 466.5229
105.	Azadirachtin N; $C_{33}H_{44}O_{15}$; 680.693460	273	NimbolidinB; $C_{38}H_{50}O_{12}$; 698.7964
106.	Azadirachtin P; $C_{33}H_{44}O_{14}$; 6 64.69406	274	Nimbolidin-E; $C_{40}H_{54}O_{12}$; 726.8496
107.	Azadirachtin Q; $C_{32}H_{40}O_{15}$; 664.651	275	NimbolinA; $C_{29}H_{38}O_9$; 530.6066
108.	Azadirachtin; $C_{35}H_{44}O_{16}$; 720.71426	276	NimbolinB; $C_{39}H_{46}O_{10}$; 674.7765
109.	Azadirachtin O; $C_{35}H_{46}O_{15}$; 706.73074	277	Nimbonolone; $C_{20}H_{28}O_2$; 300.4351
110.	Azadirachtol; $C_{28}H_{36}O_{13}$; 580.57764	278	Nimbonone; $C_{20}H_{28}O_2$; 300.4351
111.	Azadiradione; $C_{28}H_{34}O_5$; 450.56656	279	Nimbosodione; $C_{19}H_{24}O_3$; 300.3921
112.	Azadiradionol; $C_{30}H_{44}O_5$; 4 84.0	280	Nimbosone; $C_{20}H_{28}O_2$; 300.43512
113.	Azadiradionolide; $C_{28}H_{34}O_6$; 466.565	281	Nimbosterol; $C_{29}H_{50}O$; 414.7067
114.	Azadirilin; $C_{20}H_{26}O_3$; 314.41864	282	Nimocin; $C_{26}H_{34}O_4$; 410.5458
115.	Azadirin-A; $C_{20}H_{28}O_2$; 300.43512	283	Nimocinol; $C_{28}H_{36}O_5$; 452.5824
116.	Azadirin-B; $C_{20}H_{28}O_3$; 316.43452	284	Nimocinolide; $C_{28}H_{36}O_8$; 500.58064
117.	Azadirinin; $C_{39}H_{48}O_{10}$; 676.79242	285	Nimolicine; $C_{29}H_{36}O_5$; 464.59314
118.	Azadirol; $C_{32}H_{48}O_7$; 544.71932	286	Nimolicinoicacid; $C_{26}H_{34}O_6$; 442.5446
119.	Azadirolic-acid; $C_{30}H_{42}O_6$; 498.65088	287	Nimolicinol; $C_{28}H_{34}O_7$; 482.56536
120.	Azadirone; $C_{28}H_{36}O_4$; 436.58304	288	Nimolicinolic acid; $C_{26}H_{34}O_6$; 442.5446
121.	Azadironic acid; $C_{28}H_{38}O_5$; 454.59832	289	Nimolinin; $C_{20}H_{28}O_3$; 316.4345
122.	Azadironol; $C_{31}H_{42}O_6$; 522.0	290	Nimolinone; $C_{30}H_{44}O_3$; 452.6686
123.	Azadricin; $C_{19}H_{26}O$; 270.40914	291	Nimonol; $C_{28}H_{36}O_5$; 452.5824
124.	Behenicacid; $C_{22}H_{44}O_2$; 340.5836	292	Nimonolide; $C_{28}H_{36}O_7$; 484.5812
125.	Beta-asarone; $C_{12}H_{16}O_3$; 208.25364	293	Nimosone; $C_{19}H_{24}O_4$; 316.39146
126.	Beta-caryophyllone; $C_{15}H_{24}O$; 220.35046	294	N-tetradecane; $C_{14}H_{30}$; 198.388
127.	Beta-eudesmol; $C_{15}H_{26}O$; 222.36634	295	N-undecane; $C_{11}H_{24}$; 156.30826

128.	Beta-myrcene; $C_{10}H_{16}$; 136.23404	296	Ochininacetate; $C_{36}H_{42}O_8$; 602.7139
129.	Beta-nimolactone; $C_{23}H_{30}O_5$; 386.4813	297	Odoratone; $C_{30}H_{48}O_4$; 472.6997
130.	Beta-pinene; $C_{10}H_{16}$; 1 36.23404	298	OhchinolideB; $C_{35}H_{44}O_{10}$; 624.7179
131.	Beta-sitosterol; $C_{29}H_{50}O$; 414.7067	299	Oleic acid; $C_{18}H_{34}O_2$; 282.46136
132.	Bornyl acetate; $C_{12}H_{20}O_2$; 196.286	300	O-methylnimbiol; $C_{19}H_{26}O_2$; 286.40854
133.	Bornyl isovalerate; $C_{15}H_{26}O_2$; 238.36574	301	Pyronimbicacid; $C_{25}H_{28}O_5$; 408.4868
134.	Campesterol; $C_{28}H_{48}O$; 400.68012	302	Quercetin 3-galactoside; $C_{21}H_{20}O_{12}$; 464.3763
135.	Cis-muurola-4(14),5-Diene; $C_{15}H_{24}$; 204.35106	303	Quercetin; $C_{15}H_{10}O_7$; 302.2357
136.	Cis-verbenol; $C_{10}H_{16}O$; 152.23344	304	Quercitrin; $C_{21}H_{20}O_{11}$; 448.3769
137.	Cycloeucalenone; $C_{30}H_{48}O$; 424.70152	305	Rutin; $C_{27}H_{30}O_{16}$; 610.5175
138.	Cycloleucalenol; $C_{30}H_{50}O$; 426.7174	306	Salannin; $C_{34}H_{44}O_9$; 596.70776
139.	Deacetylazadirachtinol; $C_{33}H_{42}O_{14}$; 662.67818	307	Salanninolide; $C_{34}H_{44}O_{11}$; 628.7066
140.	Deacetylnimbinene; $C_{26}H_{32}O_6$; 440.5287	308	Salannol; $C_{32}H_{45}NO_9$; 587.701
141.	Decarboxynimbicacid; $C_{25}H_{30}O_6$; 426.5021	309	Salannolacetate; $C_{34}H_{47}NO_{10}$; 629.7377
142.	Demethylnimbinol; $C_{17}H_{22}O_4$; 290.3542	310	Salannolactam 21; $C_{34}H_{45}$ N O_9; 611.7224
143.	Demethylnimbionol; $C_{17}H_{22}O_4$; 290.3542	311	Salannolactame-I; $C_{34}H_{45}NO_9$; 611.7224
144.	Deoxyazadirachtolide; $C_{33}H_{48}O_7$; 556.73	312	Salannolactame-II; $C_{34}H_{45}NO_9$; 611.7224
145.	Desfurano azadiradione; $C_{24}H_{32}O_4$; 384.5085	313	Salimuzzalin; $C_{30}H_{42}NO_7$; 514.6503
146.	Desfurano-desacetylnimbin-17-One; $C_{24}H_{30}O_8$; 446.4902	314	Scopolectin; $C_{10}H_8O_4$; 192.16812
147.	Diacetylazadirol; $C_{36}H_{52}O_9$; 628.7927	315	Spathulenol; $C_{15}H_{24}O$; 220.35046
148.	Diepoxyazadiradione; $C_{28}H_{34}O_7$; 482.5654	316	Stearicacid; $C_{18}H_{36}O_2$; 284.4772
149.	Diepoxyazadirol; $C_{32}H_{46}O_6$; 526.704	317	Stigmast-4-en-3-one; $C_{29}H_{48}O$; 412.69082
150.	Dihydroazadirachtolide; $C_{33}H_{48}O_8$; 572.7294	318	Stigmasterol; $C_{29}H_{48}O$; 412.69082
151.	Dihydrogedunin; $C_{28}H_{36}O_7$; 484.5812	319	Sugiol; $C_{18}H_{24}O_2$; 300.43512
152.	Dihydromyrcenol; $C_{10}H_{20}O$; 156.2652	320	Terpinen-4-Ol; $C_{10}H_{18}O$; 154.24932
153.	Epiazadiradione; $C_{28}H_{34}O_5$; 450.56656	321	Tiglic acid; $C_5H_8O_2$; 100.11582
154.	Epoxyazadiradione; $C_{28}H_{34}O_6$; 466.56596	322	Tirucalla-7,24-dien-3beta-Ol; $C_{30}H_{50}O$; 426.71740
155.	Epoxyazadiradionebenzoate; $C_{33}H_{36}O_6$; 528.6353	323	Tirucallol; $C_{30}H_{50}O$; 426.7174
156.	Esculetin; $C_9H_6O_4$; 178.14154	324	Trans-cinnamic acid; $C_9H_8O_2$; 148.15862
157.	Ethylbutyrate; $C_6H_{12}O_2$; 116.15828	325	Trans-pinocarveol; $C_{10}H_{16}O$; 152.23344
158.	Flowerine; $C_{20}H_{20}O_6$; 356.125988	326	Triacontanol; $C_{30}H_{62}O$; 438.81268
159.	Flowerone; $C_{20}H_{20}O_6$; 356.3692	327	Vanillic Acid; $C_8H_8O_4$; 168.14672
160.	Fraxinellone; $C_{14}H_{16}O_3$; 232.27504	328	Vanillin; $C_8H_8O_3$; 152.14732
161.	Galaxolide; $C_{18}H_{26}O$; 258.39844	329	Vepaol; $C_{36}H_{48}O_{17}$; 752.7561
162.	Gallic Acid; $C_7H_6O_5$; 170.11954	330	Vepinin; $C_{28}H_{36}O_5$; 452.5824
163.	Gamma-muurolene; $C_{15}H_{24}$; 204.35106	331	Vilasinin triacetate; $C_{32}H_{42}O_8$; 554.2879
164.	Gedunin; $C_{28}H_{34}O_7$; 482.56536	332	Vilasinin; $C_{26}H_{36}O_5$; 428.561
165.	Germacrene B; $C_{15}H_{24}$; 204.35106	333	Vilasinin-1-3-diacetate; $C_{30}H_{40}O_7$; 512.6344
166.	Hexadecanoic acid; $C_{16}H_{32}O_2$; 256.42408	334	Zafaral; $C_{29}H_{42}O_5$; 470.6408
167.	Hexanal; $C_6H_{12}O$; 100.15888	335	Zeeshanol; $C_{29}H_{42}O_5$; 470.6408
168.	Hyperoside; $C_{21}H_{20}O_{12}$; 464.3763		

TABLE 2: Hit molecules from *Azadirachta indica* against basic phospholipase A_2VRV-PL-VIIIa in AutoDock.

Sl. No	Phytochemicals	Molecular formula	Molecular weight (g/mol)	Free Energy of Binding (kcal/mol)	Inhibition Constant
1.	1-Acetyl-7-tigloylnimbidinin	$C_{33}H_{44}O_7$	552.6983	-12.28	1.00 nM
2.	1-Senecioyl-3-acetylvilasininlactol	$C_{33}H_{48}O_8$	572.7294	-11.45	4.04 nM
3.	1-tigloyl-3-acetylvilasinin	$C_{33}H_{44}O_7$	552.6983	-11.24	5.75 nM
4.	24-Methylenecycloartanone	$C_{31}H_{50}O$	438.7281	-11.59	3.18 nM
5.	Cycloeucalenol	$C_{30}H_{50}O$	426.7174	-11.60	3.14 nM
6.	Cycloeucalenone	$C_{30}H_{48}O$	424.70152	-12.11	1.33 nM
7.	Kulactone	$C_{30}H_{44}O_3$	452.66856	-11.65	2.88 nM
8.	Lanosterol	$C_{30}H_{50}O$	426.7174	-11.26	5.54 nM
9.	Nimolinone	$C_{30}H_{44}O_3$	452.6686	-11.65	2.90 nM
10.	Sitosterol	$C_{29}H_{50}O$	414.7067	-11.04	8.07 nM
11.	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412.69082	-11.26	5.56 nM
12.	Stigmasterol	$C_{29}H_{48}O$	412.69082	-11.28	5.40 nM
13.	Tirucallol	$C_{30}H_{50}O$	426.7174	-11.26	5.54 nM

TABLE 3: Details of H-bond interactions of the hit molecules identified in AutoDock

H-bond details of hit molecules with the target protein Basic phospholi-pase A_2VRV-PL-VIIIa				
Sl. No.	**Compound**	**H-bonds**	**H-bonds details**	**Bond distance Å**
1.	1-Acetyl-7-tigloylnimbidinin	2	1-Acetyl-7-tigloylnimbidinin::UNK1:H66: ptn_rig:A:ALA18:O ptn_rig:A:GLY30:HN:1-Acetyl-7-tigloylnimbidinin::UNK1:O6	2.158 1.989
2.	Cycloleucalenol	1	Cycloleucalenol::UNK1:H58: ptn_rig:A:ALA18:O	2.059
3.	1-Senecioyl-3-acetylvilasininlactol	2	1-Senecioyl-3-acetylvilasininlactol::UNK1:H87: ptn_rig:A:ASP49:O ptn_rig:A:GLY30:HN:1-Senecioyl-3-acetylvilasininlactol::UNK1:O40	2.233 1.828
4.	Sitosterol	1	Sitosterol::UNK1:H67:ptn_rig:A:ALA18:O	2.216
H-bond details of hit molecules with the target protein ClassII anticoagulant phospholipase A_2				
1.	1-Cinnamoyl vilasinin lactone	1	1-Cinnamoyl vilasinin lactone:: UNK1:H61: Ptn_rig:A::GLU53:OE2	2.40
2.	Nimbocinolide	1	Nimbocinolide::UNK1:H70: Ptn_rig:A::PRO18:O	2.024
3.	2,3-dehydrosalanol	1	Ptn_rig:A:HIS48:HD1: 2,3-dehydrosalannol::UNK1:O44	1.932
4.	Azadirachtin F	2	AzadirachtinF::UNK1:H91: ptn_rig:A:TYR28:O ptn_rig:A:GLY30:HN: AzadirachtinF::UNK1:O47	2.059 1.989
5.	Nimbocinol	2	Nimbocinol:: UNK1:H55: ptn_rig:A:CYS45:O Nimbocinol::UNK1:O21: ptn_rig:A:HIS48:HD1:	1.94 2.160
6.	Epoxy-azadiradione benzoate	1	ptn_rig:A:GLY30:HN: Epoxyazadiradionebenzoate::UNK1:O23	2.881
7.	Nimbosterol	1	Nimbosterol:UNK1:H59: Ptn_rig:A:SER23:OG	2.008
8.	Tirucallol	1	Tirucallol::UNK1:H72: ptn_rig:A:ASP49:O	2.233
9.	7_Deacetyl_7_benzoylnimbinin	1	ptn_rig:A:GLY30:HN:7-Deacetyl-7-benzoylnimbinin	2.061

TABLE 4: Lead molecule identified through Rank Sum Technique using the docking scores of I-AutoDock, II- SwissDock, III-PatchDock, IV- iGemDock and V- Hex

Sl. No.	Phytochemicals	Protein	Docking Score (DST class)					
			I	II	III	IV	V	Rank Sum
1.	1-Acetyl-7-tigloylnimbidinin	Basic phospholipase A_2VRV-PL-VIIIa	4	2	4	4	2	16
2.	Cycloeucalenone		4	2	3	2	2	13
3.	Kulactone		2	2	3	2	4	13
4.	Nimolinone		2	3	3	4	1	13
5.	Cycloeucalenol		2	1	3	1	2	9
6.	24-Methylenecycloartanone		2	3	1	1	3	10
7.	1-Senecioyl-3-Acetylvilasininlactol		2	4	4	4	2	16
8.	Stigmasterol		1	1	1	1	1	5
9.	Lanosterol		1	2	2	2	2	9
10.	Stigmast-4-en-3-one		1	3	1	2	1	8
11.	Tirucallol		1	4	2	2	2	11
12.	1-Tigloyl-3-acetylvilasinin		1	1	4	4	2	12
13.	Sitosterol		1	2	2	1	4	10
14.	7-Deacetyl-7-benzoylnimbinin	ClassII anticoagulant phospholipase A_2	1	1	2	3	1	8
15.	Stigmasterol		3	2	1	2	1	9
16.	2-3-Dehydro-salannol		3	3	2	2	3	13
17.	AzadirachtinF		2	1	3	4	4	14
18.	Stigmast-4-en-3-one		1	4	2	1	3	11
19.	Cycloeucalenone		1	3	1	1	1	7
20.	1-Cinnamoylvilasinin lactone		1	3	4	4	4	16
21.	Limocinin		1	3	4	3	3	14
22.	Nimolinone		1	2	3	2	2	10
23.	Nimbocinol(7-deacetylazadiradione)		1	3	1	3	1	9
24.	Epoxyazadiradionebenzoate		1	3	2	2	3	11
25.	7alpha-senecioyl-7-deacetyl-23-O-methylnimocinolide		1	4	3	3	4	15
26.	Kulactone		1	2	2	2	2	9
27.	Nimbosterol		1	4	1	2	2	10
28.	Tirucallol		1	2	3	2	3	11
29.	Nimbocinolide		4	2	2	2	1	11

TABLE 5: Selected hit molecules from *Azadirachta indica* against class II anticoagulant phospholipase A_2 in AutoDock

Sl. No	Molecule	Molecular formula	Molecular weight (g/mol)	Free Energy of Binding (kcal/mol)	Inhibition Constant
1.	1-Cinnamoyl vilasininlactone	$C_{35}H_{44}O_7$	576.7197	-10.54	18.86 nM
2.	2-3-Dehydro-salannol	$C_{32}H_{42}O_8$	554.6711	-12.52	663.06 pM
3.	7-Alpha-senecioyl-7-deacetyl-23-O-methylnimocinolide	$C_{32}H_{40}O_8$	552.6552	-10.27	29.61 nM
4.	7-Deacetyl-7-benzoylnimbinin	$C_{32}H_{36}O_6$	516.6246	-10.06	42.49 nM
5.	AzadirachtinF	$C_{32}H_{44}O_{12}$	620.6846	-12.07	1.43 nM
6.	Cycloeucalenone	$C_{30}H_{48}O$	424.70152	-10.63	16.07 nM
7.	Epoxyazadiradionebenzoate	$C_{33}H_{36}O_6$	528.6353	-10.31	27.57 nM
8.	Kulactone	$C_{30}H_{44}O_3$	452.66856	-10.16	35.95 nM
9.	Limocinin	$C_{32}H_{42}O_5$	506.6729	-10.54	18.73 nM
10.	Nimbocinol	$C_{26}H_{32}O_4$	408.5299	-10.45	21.79 nM
11.	Nimbocinolide	$C_{28}H_{36}O_6$	468.5818	-14.44	25.87 pM
12.	Nimbosterol	$C_{29}H_{50}O$	414.7067	-10.12	38.38 nM
13.	Nimolinone	$C_{30}H_{44}O_3$	452.6686	-10.46	21.66 nM
14.	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412.69082	-10.67	15.12 nM
15.	Stigmasterol	$C_{29}H_{48}O$	412.69082	-13.28	184.62 pM
16.	Tirucallol	$C_{30}H_{50}O$	426.7174	-10.08	41.19 nM