IN SILICO EVALUATION OF ANTI-COBRA VENOM ACTIVITY IN SELECTED FRUIT CROPS

By

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(2012-09-106)

Thesis

Submitted in partial fulfilment of the

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2017

DECLARATION

I hereby declare that this thesis entitled **"*In silico* evaluation of anti-cobra venom activity in selected fruit crops"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.

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Greeshma A. S.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
3D	Three dimensional
2D	Two dimensional
DST	Dampster-Shafer Theory
et al.	And others
HTS	High-throughput screening
Kcal	Kilo calorie
M	Molar
mL	Micro litre
mM	Milli molar
nM	Nano molar
Da	Dalton
mg	Milli gram
NCBI	National Center for Biotechnology Information
PDB	Protein Data Bank
viz.	Namely
WHO	World Health Organization
µg	Micro gram
µL	Micro litre
Via.	By way of
PLA2	Phospholipase A2
CBT	Cobrotoxin
CA	Cobramine A
CB	Cobramine B
CYT 3	Cytotoxin 3
LAAO	L- Aminoacid oxidase
LN1	Long neurotoxin 1
LN2	Long neurotoxin 2
LN3	Long neurotoxin 3
LN4	Long neurotoxin 4
LN5	Long neurotoxin 5
SP	Serine protease
PRT	Proteolase
ACE	Acetylcholine esterase
HB	Hydrogen Bond

INTRODUCTION

1. INTRODUCTION

Snakebite is a serious health problem mainly in tropical and subtropical countries, where people in rural areas mainly engaged in traditional agricultural works are majority of the victims. Due to the under privileged condition and lack of proper medical facilities, the snakebite victims in these areas depend on traditional herbal medicines without documenting their treatment details. Therefore, the actual number of global burden of snakebite is unknown. However, based on the available information it is estimated that globally ~54,00,000 snakebites with 2,50,000 envenomation and around 1, 25,000 fatalities take place annually. Most snake bites and fatalities occur in Asia, Southeast, and sub Saharan Africa. India has the highest number of mortality due to snakebites (Kasturiratne *et al.*, 2008). Considering the annual high death rate, WHO had included snakebite in the list of tropical neglected diseases in 2009, though it is not a pathogenic infectious disease. Globally ~3000 snake species have been reported, of these, only 500 of them are venomous. In India, out of the 52 venomous snake species reported only five species *viz. Ophiophagus hannab* Cantor (king cobra), *Naja naja* L. (common cobra), *Daboia ruselli* Shaw & Nodder (Russell's viper), *Bungarus caeruleus* Scheider (Krait) and *Echis carinatus* Scheider (Saw scaled viper) cause majority of the mortality. Among these, king cobra is not common throughout India.

Immunotherapy is the only treatment against snake envenomation in modern medicine which was discovered by Calmette in 1894 and is still followed with little modifications in the method of anti-venom preparation. First, venom is milked from the snake by mechanical pressure on the venom gland, then diluted and injected into a horse or goat for immunising the animal. As the animal build up immunity to the venom, the dosage is increased for creating antibodies rich blood. Ten to fifty injections during 3-15 months is necessary for valid immunisation of the animal. The immunised animal serum is tested, isolated, purified and preserved for further use. As the animal serum is used for inducing immunity in human being and it may induce

series side-effects to human body. It's high cost, non-availability, lack of storage facility in rural areas and difficulty in identifying the snake species are other limitations in immunotherapy. Recently monovalent anti-venom against different snake species is available but due to the difficulties in identifying the snake species still polyvalent anti-venom has been used. In India the polyvalent anti-venom is prepared by injecting the venom of four venomous snake species in equal dose to the animal and the administration of such polyvalent antivenom may induce series complications to the patients.

Many plants, animals and microorganisms produce toxic materials for defensive or food capturing purpose and such molecules are evolved through long term evolutionary process. In animals these molecules may be secreted by specialized cells or in specialized venom apparatus/gland. Snake venom is produced by specialized glands and delivered through specialized envenomation system. Further, venom must be introduced (injected) into recipient tissues in order for deleterious effects to occur, while poisons are typically ingested. The toxic substances produced by specialized gland and injected into other animals are called as venomous and the toxic substances secreted by specialized tissues which induce toxicity when spread over other animals are termed as poisonous. The venom may be simple or a complex mixture of secretary products. Snake venom is a gold mine of many bioactive molecules that includes 90% of proteins and remaining 10% consists of nucleotides, inorganic ions etc. Many of these proteins are harmless and pharmaceutically important (Nisha *et al.*, 2010). Over 62 pharmacologically active molecules have been reported from the venom of various snake species and several drugs available in the market are derived from snake species. For examples, captopril/enalapril isolated from the *Bothrops jaracusa* (Brazilian arrow head viper) venom is used for the treatment of high blood pressure, integrilin (eptifibatide) derived from *Sisturus miliarus barbouri* (South Eastern pigmy rattlesnake) used as platelet aggregation inhibitor, ancrod (viprinex) isolated from *Agkistrodon rhodostoma* (Malayan pit viper) venom is used as fibrinogen inhibitor and stroke. Generally, 20 different types of toxic enzymes are found in venomous snake

species, of these, 12 of them such as phospholipase A2, L- amino acid oxidase, phosphodiesterase, 5'-nucleotidase, phosphomonoesterase, deoxyribonuclease, ribonuclease, adenosine triphosphatase, hyaluronidase, NAD nucleosidase, arylamidase and peptidase are common in most of the snake species (Raweerith *et al.*, 2005). The quantity and quality of snake venom may vary from species to species, age and geographical condition.

In modern medicine, drug molecules are developed based on the principle that a single drug may act on specific site of a single target while plant derived herbal extract contains a plethora of phytomolecules and many natural compounds can simultaneously act on more than one target. Therefore, now-a-days the physicians prescribed several commercially available herbal medicines for the disease like stomach problems, which may cause due to multi-factorial causation. The plant derived molecules are synthesized within the living system and interacted with other bio-molecules or chemical constituents in the biological system, and ultimately evolved as non-toxic new chemical entities with pharmaceutical properties. The efficacy of such molecules have been tested, modified and evolved through long-term evolutionary process and they can be used as safe drugs, which may cause lesser or no side effects (Sreekumar, 2016). Since time immemorial, plants have been used as a best and renewable source of medicine. It can produce innumerable number of secondary metabolites using raw materials formed during the primary metabolic pathways in response to needs and challenges of its environment. The occurrence of these secondary compounds amounts alkaloids as 20%, flavonoids as 15%, triterpenes and simple phenolics around 10% and others in very low level, with limonoid being the least. Most of these have various applications in the area of pharmaceuticals, food additives, fragrances, pesticides, etc. About 600,000 secondary compounds from plants have been already reported. Plants are also the best synthesizer of nanoparticles having pharmaceutical properties, which are used for site targeted drug delivery to treat disease like cancer (Sreekumar, 2016). They are always synthesizing novel compounds with multi-therapeutic activity and many plant derived drugs widely used in modern

medicine could not be synthesized economically and most of these drugs synthetic pathways remain as unknown. Thus, despite the advancement in organic chemistry plants are still continue as a best organic chemist in the world. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. About 125 such plant derived compounds are currently in use as drugs and 25% of modern prescription drugs contain at least one compound derived from higher plants.

In Indian traditional systems of medicine especially in Ayurveda remedies are available to treat all diseases so far reported. India has rich repository of biodiversity and ranks top fourth position in floral diversity among Asian countries with 33% endemic species and showed high genetic diversity among inter and intra species level, in which high value low volume phytomolecules with diverse pharmaceutical properties are locked. However, the efficacies of such molecules are seldom isolated and validated scientifically and the effort to find out novel molecules with pharmaceutical properties is still hampered. It is mainly because of several reasons such as high investment, lack of raw materials, screening methodologies are time consuming and yielded less number of lead after high investment etc. (Nisha *et al.*, 2014).

Snake venom is a complex mixture of toxic proteins and development of a single medicine against it following the principle of modern medicine may be a herculean task. Globally 600 plant species have been used against snakebite and in India about 350 plant species are known as antidote to snake venom. But the efficacy and molecular mechanism of drug action of these plants are seldom investigated. It is well acknowledged that *in silico* screening coupled with *in vitro* and *in vivo* screening is the best method to demonstrate the drug activity and identification of lead molecules from plants (Shefin *et al.*, 2016).

Perusal of the literature revealed that the fruit yielding plants *Punica granatum* (L.), *Syzygium cumini* (L.), *Mangifera indica* (L.), *Tamarindus indica* (L.) and *Phyllanthus emblica* (L.) have been used to treat snake bite in Indian traditional system of medicine. However, its efficacy and phytomolecules responsible for antidote activity are not scientifically demonstrated. In these backdrops, the present investigation was undertaken with following objectives

To evaluate cobra (*Naja naja* L.) venom detoxification activity and identification of potential lead molecules in common five fruit crops *viz.* *Punica granatum* (L.), *Syzygium cumini* (L.) *Mangifera indica* (L.), *Tamarindus indica* (L.) and *Phyllanthus emblica* (L.) using bioinformatics tools.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 SNAKEBITE AND ITS IMPACTS

Snake envenomation is one of the global serious health concerns since time immemorial wherever venomous snakes are present. Snakes are present in all over the world except in Polar Regions. High rate of envenomation and subsequent mortality and morbidity reported in the rural areas of tropical countries like India, South Africa, etc. where agriculture is the main stream of economy and majority of the people are engaged in field oriented agricultural works (Mohapatra *et al.*, 2011). It is also noted that majority of the snakebite victims depend on traditional healers and they are not properly documenting the victims' details. Therefore, estimating the actual snakebite mortality rate is not possible and based on the available information from hospital records many authors have estimated the snakebites death rate (Nisha *et al.*, 2014). Williams *et al.* (2010) estimated that the annual death rate due to snake venom between 5.4-5.5 million globally and 20,000-1, 25,000 in India. This is six times greater than the death caused due to neglected tropical diseases and therefore the World Health Organization included snakebite along with the neglected tropical diseases in 2009. In addition, around three times as many amputations and other permanent disabilities are caused by snake bites annually. Globally highest rate of snakebite death recorded in India, about 45,900. The snake bites and its impacts in humans are well reviewed by many authors (Mohapatra *et al.*, 2011).

2.1.1 Snakes and venom composition

Approximately 3000 species of snakes are reported worldwide, of these, 500 species are venomous (Jagtap *et al.*, 2016). Of the 256 species reported from India, 52 species are venomous (Rana *et al.*, 2015). Although majority of snake species are non-venomous and typically kill their prey with constriction rather than venom, venomous snakes (15% out of 3000 known species) 5-7 are reported to be found on every continent except Antarctica (Kamal *et al.*, 2014). In India, maximum mortality and morbidity are caused by the "Big 4" species i.e., the Indian cobra (*Naja naja*), the

common krait (*Bungarus caeruleus*), the Russell's viper (*Daboia russelii*) and the saw-scaled viper (*Echis carinatus*). The king cobra (*Ophiophagus Hannah)* also cause death but it is not common (Makhija *et al*., 2010). Highly venomous seven coral snakes and 29 sea snakes (Rana, 2015) have also been reported from India. These venomous snakes are included in four families, *viz.* Atractaspididae, Elapidae, Hydrophidae and Viperidae. The major three families in the Indian subcontinent are Elapidae which includes common cobra, king cobra and krait and Viperidae which includes Russell's viper, pit viper and saw-scaled viper and Hydrophidae (sea snakes). Indian Cobra (*Naja naja*) is also known as spectacled cobra causes highest mortality rate in India. The Indian cobra produces systemic poisoning because of the rapid action of neurotoxin which causes respiratory paralysis and death (Banerjee, 1978). It is distributed in India, Sri Lanka, Pakistan, Nepal and Bangladesh. In Elapids the venom glands are present behind the eye and are surrounded by compressor muscles. They inject venom into the prey with the fangs which are modified teeth. In Elapids, the short fangs are mounted on a relatively fixed maxilla in front of the mouth. In humans, snakes usually inject venom subcutaneously or intramuscularly and the average dry weight of venom injected at a strike by Indian cobra is approximately 60 mg (Kamal *et al*., 2014).

Snake venom is merely modified saliva with highly complex cocktail of proteins, peptides, non-protein toxins, carbohydrates, lipids, amines and other molecules. The chemical composition of venom differs from one taxonomic level to other. Further, the composition may vary between snakes in different geographical locations. The composition of venom in each snake may change based on diet, age, season and environment. About 90% of the dry weight of the venom consists of proteins. There are more than hundred different proteins in each venom; with elapid and viperid venoms constituting 25-70% and 80-90% of enzymes respectively (Gupta and Peshin, 2014). The snake venoms are mainly classified as neurotoxic and hemotoxic. No snake venom contains just one type of toxin. Neurotoxic venoms act

by disrupting the neuromuscular junctions and limit while hemotoxic venoms destruct tissue systems besides their effect on circulatory system. Serine proteases stimulate blood clotting. Widespread damage to mitochondria, skeletal muscle, vascular endothelium, red blood cells, leucocytes, platelets, peripheral nerve endings, and other membranes is caused due to phospholipase A2, the most common enzyme present in the venom. Most of the cobra venoms contain acetylcholinesterase enzyme that cause tetanic paralysis. Presynaptic neurotoxins release acetylcholine at the nerve endings at neuromuscular junctions and damage the endings, interfering with its release (Gupta and Peshin, 2014). The details of venom components and the list of pharmacologically active compounds isolated from snake venom as well as drug molecules derived and commercialized from snake venom are well reviewed (Nisha *et al.*, 2010).

2.1.2 Snake bite treatment

Antivenom immunotherapy is the only specific treatment against snake venom in modern medicine since its discovery in 1894 by Calmette. In India anti snake venom (ASV) is formulated against the four common species of snakes. ASV is administered only in patients with clear evidence of systemic envenoming or the one with local envenomation. Generally, administration of 8-10 vials of ASV is recommended and further dosing depends on response to the initial dose (Gupta and Peshin, 2014). Anti-venoms are produced through fractionation of plasma obtained from immunized animals, mainly horses. The anti-venom produced can be either monovalent or polyvalent which depend up on the number of species whose venoms are used for immunization. Although efficiency of monovalent anti-venom is more, the production of polyvalent anti-venom is preferred in many countries since identification of snake species is generally not possible for the physician. Traditionally, the production has focused on four species believed to be responsible for most deaths: *Naja naja*, *Bungarus caeruleus*, *Daboia russelii* and *Echis carinatus*. However, a number of other species that contribute morbidity and mortality is not considered, and envenoming by these species using existing anti-venoms usually does not respond (Kamal *et al.*, 2014).

In fact, majority of the snake bite victims are in rural areas who are engaged in field oriented agriculture related job and they depend on herbal medicines. It is believed that herbal medicines have no side effects or fewer side effects, cost effective and need not require sophisticated storage system when compared to the antivenom therapy.

2.1.3 Limitation in anti-venom therapy

Anti-venom immunotherapy is the only specific treatment since its discovery in 1894 by Calmette. The major disadvantages of anti-venom therapy are;

- Due to the action of non-immunoglobulin protein present in commercially available anti-venom, several side effects to human body such as anaphylactic shock, pyrogen reaction and serum sickness are observed.
- Local hemorrhage, necrosis and tissue damage are seen due to the failure of anti-venom to neutralize the low molecular weight, less immunogenic toxic components present in venom.
- Anti-venom raised against the venom of a snake from a particular geographical origin may not be able to neutralize envenomation by snakes from other geographical locations.
- Lack of availability of sufficient amount of quality venom and storage facility in rural areas.
- Due to the difficulty in identifying the snake species, instead of using monovalent type, polyvalent type anti-venom is commonly used, which may be hazardous to the patient and is less effective (Nisha *et al.*, 2014).

2.2 COBRA VENOM PROTEINS

Cobra venom is a gold mine of various biomolecules and 14 toxic proteins were selected as target. Among these PLA 2 was a major component which induce lethality. It catalyzes the hydrolysis of fatty acid esters at second position of 1, 2-diacyl-sn-phosphoglycerides which require Ca^{2+} ion as the cofactor. PLA 2 have wide range of pharmacological activity such as neurotoxicity, myotoxicity, cardiotoxicity, anticoagulant, hemolytic, hemorrhagic, platelet aggregation and edema inducing

activity (Doley *et al.*, 2010). His47 and Asp90 were marked as the residue of this enzyme. ASP48 is preceded by His47 and to this Asp48 residue, Ca^{2+} ion bind during catalytic reaction. Thus ASP48 was taken as the critical residue. Cobrotoxin is the next main neurotoxin found in cobra venom. It consists of 62 amino acids stabilized by disulphide bridges. Due to the presence of epsilon- amino group of Lys47 and guanidine group of Arg33, the toxin has high neuromuscular blocking activity (Yang, 1999). Arg33 was taken as the critical residue. The venom also contains several long and short neurotoxins belonging to the three finger protein family. Neurotoxins are nerve poisons which acts directly on neurons and interferes the function of membrane proteins and ion channels, therefore counteracting nerve impulses to induce muscle contraction and lead to cessation of breathing, paralysis and death (Du, 2002). Structural information about LN 1, LN 2, LN 3, LN 4 and LN 5 were available in SWISS-MODEL repository. Cytotoxins are another class of proteins under 3 finger protein family. They constitute about 60% of dry weight of the cobra venom (Nisha *et al.*, 2014). They cause lethality, cytotoxicity, contraction of muscle, activation of PLA 2, platelet aggregation etc. There are 60 amino acids in this protein. PRT enzyme is the key enzyme responsible for proteolysis or degradation of proteins. SP is the next enzyme, which have a serine residue as its active residue which can interact with positively charged amino acids and cleaves the proteins. It also catalyzes wide range of reactions like fibrinolytic, endothelial cells and blood platelets. The selected active residue was Ser31. The catalysis of L-amino acids to oxoacids is done by LAAO enzyme present in the cobra venom. Structural analysis of these enzymes revealed the presence of a dynamic active site and 3 domains like FAD binding domain, a substrate binding domain and a helical domain. This enzymes performs pharmacological effects like platelet aggregation, hemorrhagic effect, cytotoxicity, anticoagulant etc. (Tan and Fung, 2010; Philips *et al.*, 2010). The last one is the acetylcholinesterase enzyme which degrades neurotransmitter acetylcholine at the neuromuscular junction. Thus it terminates synaptic transmission (Ahmed *et al.*, 2009).

2.3 IMPORTANCE OF HERBAL DRUGS AGAINST SNAKE BITE

Since time immemorial man depend on plants for curing diseases and healing accidental wounds. They way in which the use of plants as medicine adopted through trial and error methods and practicing well are termed as traditional medicine. This knowledge system is referred as traditional or ethnomedicine. In India, the forgoing knowledge system is documented in vedic literature such as Shusruta and Charak Samhita. India has rich biodiversity and the plant and animal derived products have been used extensively in Ayurvedic system of medicine since several millennia (Sreedevi *et al.*, 2013). According to Sahu *et al.* (2017) the tribal areas the man-plant relations are better tuned and are the major centers of ethnomedicinal wisdom. According to the 'All India Coordinated Project on Ethnobiology' sponsored by the Ministry of Environment and Forest, New Delhi, 40% of 16,000 recorded flowering plants in India have ethnomedicinal value, but only 10% are used by drug and pharmaceutical industries. The intrinsic importance of these medicinal plants serve as a potential source of new drugs (Pushpangadan, 2005). The tribal people in India not only depend upon forests for their livelihood but also depend on traditional medicine as a primary healthcare source. There are about 550 tribal communities covered under 227 ethnic groups residing in India and there are about 5000 villages in different forest. In the developed countries 25% of the medical drugs are based on plants and their derivatives.

Numerous plant species have been used as folk medicine for treating snake bite in almost all parts of the world, where venomous snakes occur. Application of the plant or its sap onto the bitten area, chewing leaves or barks or drinking plant extracts or injecting the extracts are some procedures intended to counteract snake venom activity (Alam *et al.*, 2016). In Ayurvedic medicine, the roots of the plant *Ophiorrhiza mungo, Peristrophe bicalyculata, Gymnema sylvestre Gloriosa superba, Cucumis colosynthis, Alangium salvifolium,* leaves of *Enicostemma axillare,*

Calycopteris floribunda, Calotropis gigantea, Aristolochia indica are used against snake venom. In ayurveda species specific usage of the medicinal plants against snake bites is also reported. For examples, root extract of *Abrus precatorius* is used against krait bite, leaf paste of *Azadirachta indica* with rock salt is used against viper bites. *Aristolochia indica* is used widely against snake bite. *Achyranthes aspera*, is used in treatment of bleeding, renal complications, scorpion bite, snake bite etc. Knowles was the first one who scientifically investigated about herbal antidotes. Knowles screened several plant constituents which are used by local healers, but he failed to find their activity against snake envenomation, either due to sub lethal dose of venom or non-lethal dose. Later, Mhaskar and Caius challenged the effectiveness of herbal antidotes by screening 314 plants and 184 combinations against venom induced lethality. This pioneering theory was later contradicted by various reports on effectiveness of herbal antidotes against systemic toxicities as well as lethality (Alam *et al.*, 2016; Knowles *et al.*, 1921; Mhaskar and Caius, 1931). Preliminary investigations have suggested that pathophysiological changes induced due to snake venom can be neutralized by several classes of constituents such as steroids, terpenoids, alkaloids and glycosides derived from plants (Martz, 1992; Mors *et al.*, 1989; Mors *et al.*, 2000; Silva *et al.*, 2004; de Almeida *et al.*, 2004; Fatima and Choudhary, 2004; Houghton and Osibogun, 1993). More than 800 plant species have been screened for the anti-snake venom activity, however, very few plant extracts have shown significant protection against snake-venom and none of the pure isolates has displayed equivalent activity (Alam and Gomes, 1998; Fatima and Choudhary, 2004; Mahanta and Mukherjee, 2001; Alcaraz and Hoult, 1985).

2.4 DRUG DISCOVERY FROM PLANTS

For millennia, the major source of therapeutic agents is medicinal plants, and still many of today's drugs are plant derived natural products or their derivatives (Kinghorn *et al.*, 2011; Newman and Cragg, 2012). The first written records on medicinal applications of plants were dated on 2600 BC. It also reports about the existence of

a plant based medicinal system in Mesopotamia, comprising about 1000 plant derived medicines (Borchardt, 2002; Cragg and Newman, 2013). Rational drug discovery from plants started at the beginning of the 19th century. In 1805, morphine became the first pharmacologically active compound isolated in pure form from a plant *Papaver somniferum*. The German pharmacist Friedrich Serturner succeeded in isolating the analgesic and sleep inducing agent from papaver plant and he named it as morphium (morphine), the Greek god of dreams, Morpheus. He published a comprehensive paper on its isolation, crystallization, crystal structure, and pharmacological properties, which he studied first in stray dogs and then in self-experiments (Serturner, 1817). This invention triggered the examination of other medicinal herbs, and during the following decades of the 19th century, many bioactive natural products, primarily alkaloids (e.g., quinine, caffeine, nicotine, codeine, atropine, colchicine, cocaine, capsaicin etc.) could be isolated from their natural sources (Corson and Crews, 2007; Hosztafi, 1997; Kaiser, 2008; Zenk and Juenger, 2007). Salicylic acid was the first natural compound produced by chemical synthesis in 1853 (Kaiser, 2008). Salicylic acid is derived from bark of willow tree. Plant derived natural compounds can be predominantly found in the field of anti-cancer agents, e.g., paclitaxel and its derivatives from yew (*Taxus*) species, vincristine and vinblastine from Madagascar Periwinkle (*Catharanthus roseus* (L.), and camptothecin and its analogs initially discovered in the Chinese tree *Camptotheca acuminata* Decne. (Cragg and Newman, 2013; Kinghorn *et al.*, 2011).

The major disadvantage is that in most of the cases, when a plant becomes commercialized as a herbal medicine or when one of its constituents starts getting used as a pharmaceutical drug, its populations become threatened due to extensive wildcrafting and unsustainable harvesting techniques (Cordell, 2011; Vines, 2004). Plant extracts contains a plethora of chemical. Moreover, the plant derived compounds are synthesized within the living system by repeated biological testing through evolutionary process and therefore they are safe and induce less or fewer side effects

than synthetic drugs (Sreekumar *et al.*, 2014). Perusal of the literature revealed that globally about 600 plant species and in India ~350 plant species have been used against snake envenomation (Deepa *et al.*, 2016). However, its efficacy and molecular mechanism of drug action are seldom scientifically demonstrated. In the light of these, validation of the efficacy of herbal drugs used against snakebite attains prima face importance.

Perusal of the literature indicate that of the 252 drugs considered as basic and essential by the World Health Organization, 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. About 125 such plant derived compounds are currently in use as drugs and 25% of modern prescription drugs contain at least one compound derived from higher plants (Rates, 2001). Generally, natural products particularly plant derived drug molecules have greater number of chiral centers, increased steric complexity, higher number of oxygen atoms, lower ratio of aromatic ring atoms to total heavy atoms, higher number of solvated hydrogen bond donors and acceptors, grater molecular rigidity and broader distribution of molecular properties such as molecular mass, octanol water partition coefficient, and diversity of ring systems (Koehn and Carter, 2005). These characteristic features attributed enormous structural and chemical diversity and about 40% of the chemical scaffolds found in natural products are not in today's medicinal chemistry (Lahlou, 2013). Of the 1073 new chemical entities (small molecules) approved between 1981 and 2010 only 36% purely synthetic and over 50% natural products, majority from higher plants. About 78% of antibacterial and 74% of anticancer compounds are natural products or have been derived from or inspired by natural products. In addition to drug molecules plants are also synthesizing nanoparticles which can be effectively used for site targeted drug delivery. These all well reviewed by many authors (Sreekumar *et al.*, 2016).

2.4.1 Importance of nutraceuticals

The word nutraceuticals is comprised of two word 'Nutrient' and 'Pharmaceuticals'. Nutraceutical means any non-toxic food component that has scientifically proven health benefits including prevention and treatment of disease (McAlindon, 2006). The term 'nutraceutical' was coined from nutrition and pharmaceutical in 1989 by Stephen Defelice (Stephen, 1995). In India a large number of populations depend on natural and alternative medicine due to the imbalance and deficiencies in national medical delivery system. India's food market in functional foods and beverages forecast to account for almost 71% of the dietary supplement sector in 2017. Nutraceuticals are commercialised in the form of capsule, tablet or powder in a prescribed dose while modern nutraceuticals are available as forms of food such as probiotic drink and yogurt. The primary set of rules governing the nutraceutical market is assigned by the Dietary Supplement Health and Education Act (DSHEA) which was passed in the year 1994 (McAlindon, 2006).

2.5 DRUG DISCOVERY PROCESS

Developing a new drug from an idea to the launch of a finished product is a complex and cumbersome process which can take about 12–15 years and cost in excess of $1 billion. The need for new drug discovery occur when there is a disease or clinical condition without a suitable medical products. The initial stage of drug discovery is to gather the information regarding the disease and its pathways involved which is under academic or research field. The outcome of this activity is the identification of a target which may require further validation prior to progression into the lead discovery phase in order to justify a drug discovery effort (Huges *et al.*, 2011). After the discovery of a lead molecule, it has to undergo preclinical trials, then clinical trial which include

different phases and finally has to be approved by Food and Drug Administration. The different stages of drug discovery process are schematically depicted below

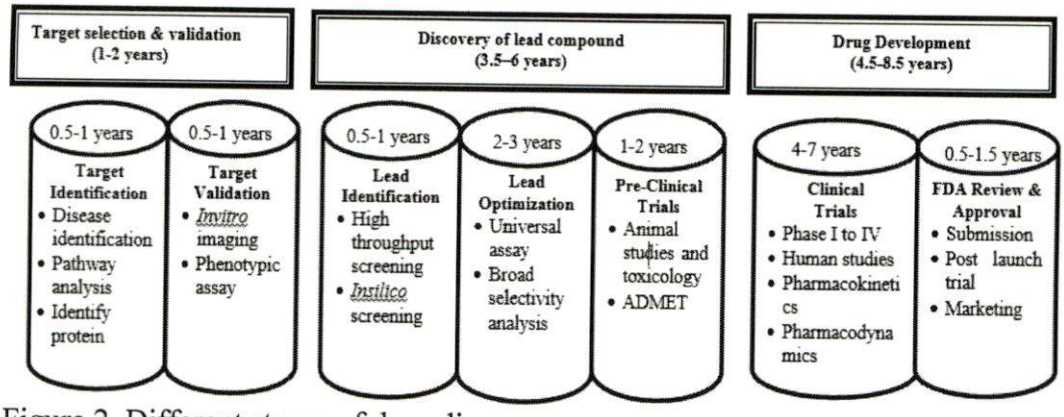


Figure 2. Different stages of drug discovery process.

2.6 METHODS FOR SCREENING PLANT DERIVED MOLECULES FOR DRUG DISCOVERY AND ITS LIMITATION

During the past few years, the design and development of new screening assays in drug discovery has become increasingly important. Since the disease rate is increasing rapidly, the demands for screen more targets with higher throughput and more information content in combination with the affordable costs also increased. To meet these demands, a great deal of research is required in areas such as target selection and in the development of improved methodologies for detection and cell-based screens. Different screening methods for lead identification are given below

Table 1. Different screening methods.

Screening methods	Description
High throughput screening	Large numbers of compounds are analyzed in an assay generally designed to run in plates of about 384 wells and above. Large compound collections often run by big pharma company but smaller compound banks can also be run in either pharma or academia which can help reduce the costs (Huges *et al.*, 2011)
Focused screening	Target-focused compound libraries are collections of compounds which are designed to interact with an individual protein target or with a family of related targets (such as kinases). They are used for screening against therapeutic targets in order to find hit compounds that will be further developed into drugs (Haris *et al.*, 2011).

Fragment screening	In this method, a number of small molecules or fragments, typically with molecular weight less than 300 Da and with low affinities, are evaluated for specific interaction with the target. Soak small compounds into crystals to obtain compounds with even milli molar activity which can then be used as building blocks for larger molecules (Huges *et al.*, 2011).
Structural aided drug design	Structure based drug design generally uses crystal structures to help design molecules. It is designed in such a way that it directs the discovery of a drug lead, which is not a drug product but, specifically, a compound with at least micro molar affinity for a target protein molecule (Anderson, 2003).
Virtual screening	Virtual screening defines the use of the information in the creation of computational models or simulations that can be used to make predictions, suggest hypotheses, and ultimately provide discoveries or advances in medicine and therapeutics (Ekins *et al.*, 2007).
Physiological screening	A tissue-based approach is designed for the determination of the effects of a drug at the tissue rather than the cellular or subcellular level, for example, muscle contractility (Huges *et al.*, 2011).

2.7 APPLICATION OF BIOINFORMATICS IN DRUG DISCOVERY PROCESS

The National Centre for Biotechnology Information defines Bioinformatics as "Bioinformatics is the field of science in which biology, computer science, and information technology merge into a single discipline." There are mainly three sub divisions in Bioinformatics. They are

- The development of new algorithms and statistics which is used to assess relationships among members of large data sets.
- The analysis and interpretation of various types of data. This includes nucleotide, amino acid sequences, protein domains, and protein structures.
- The development and implementation of new tools that helps to access and manage different types of information gathered through these biological experiments (Kanaujia, 2004).

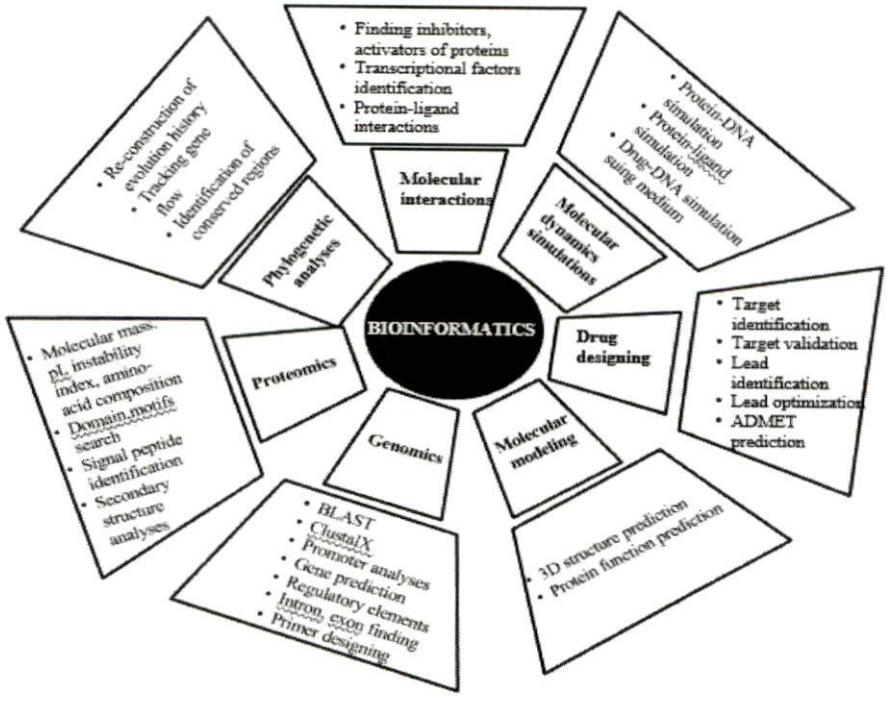


Figure 3. Bioinformatics in different field.

2.8 *IN SILICO* SCREENING AND IDENTIFICATION OF LEAD MOLECULES FROM PLANTS

Drug discovery and development are very time consuming and expensive processes. There is an ever growing effort to apply computational power in order to streamline drug discovery, design, development and optimization. In biomedical arena, computer aided or *in silico* design is being used to facilitate hit identification, hit-to-lead selection, optimize the absorption, distribution, metabolism, excretion, toxicity profile (ADME/Tox), and avoid safety issues. Different computational approaches include ligand-based drug design, structure based drug design and quantitative structure-activity relationships. In the early stage of drug discovery, structure-based design method is widely used to optimize these leads into drugs (Singh *et al.*, 2006). This rational approach is severely limited to target proteins that are amenable to structure determination. Although the protein data bank (PDB) is growing

rapidly (~13 new entries daily), the 3D structure of only 1 to 2% of all known proteins has as yet been experimentally characterized (Berman *et al.*, 2006). However, advances in sequence comparison, fold recognition and protein modelling algorithms have enabled more about protein structure information to homologous proteins.

2.8.1 Preparation of target

A target is a broad term which is applied to a range of biological entities which may include for example proteins, genes and RNA. A good target needs to be efficacious, safe, meet clinical and commercial needs and, above all, be 'druggable'. A 'druggable' target is accessible to the putative drug molecule, be that a small molecule or larger biologicals and upon binding, elicit a biological response which may be measured both in vitro and in vivo (Huges *et al.*, 2011). Data mining of available biomedical data regarding a particular disease has led to a significant increase in target identification process. In this context, data mining refers to the use of a bioinformatics approach to not only help in identifying but also selecting and prioritizing potential disease targets (Yang *et al.*, 2009). Three dimensional Structure of various target protein can be directly downloaded from various databases like RCSB Protein Data Bank, UniProt etc. If the structure is not available on any database, it can be modelled through Modeller or can be modelled online through Homology modelling.

2.8.2 Preparation of ligand

Ligand details can be retrieved from various databases. Some of the databases which provide details about ligand are PubChem, ChemSpider, FooDB etc. The three dimensional structure of ligand can be retrieved from CORINA. PubChem has archived 35.6 million of unique chemicals. Among them, 25.3 million satisfy the rule of five, 1.85 million have been tested in at least one bioassay, and 0.8 million have been reported as active (Wang *et al.*, 2013). Ligand structure can also be drawn with the help of software like ChemSketch.

2.8.3 Docking

Molecular docking has become an increasingly important tool for drug discovery (Meng *et al.*, 2011). Since 1980, molecular docking is the most common method which has been widely used (Kuntz *et al.*, 1982). In pharmaceutical industries, programs based on different algorithms to perform molecular docking studies have played an important role. Various excellent reviews on docking have been published in the past and many comparison studies were conducted to evaluate the relative performance of the programs (Brink *et al.*, 2009; Cross *et al.*, 2009; Plewczynski *et al.*, 2011). The docking process involves mainly two basic steps. They are, prediction of pose of the ligand and assessment of the binding affinity. These two steps are related to sampling methods and scoring schemes, respectively. Knowing the location of the binding site before docking processes significantly increases the docking efficiency (Goodford, 1985; Kastenholz *et al.*, 2000; Levitt and Banaszak, 1992; Laskowski, 1995; Glaser *et al.*, 2006; Brady, 2000; Mezei, 2003).

DOCK 1.0 was the first automated receptor ligand docking program. It was design in 1982 by Irwin Kuntz in The Department of Pharmacology at The University of California at San Francisco (Kuntz *et al.*, 1982). At present there are at least a dozen docking tools available in the market, the most commonly used being: AutoDock, DOCK, FlexX, GOLD, LigandFit and the relatively new tools are Glide, FRED and the youngest Surflex (Goodsell and Olson, 1990; Friesner *et al.*, 2004).

2.8.4 AutoDock

AutoDock is a freely available software that combines an empirical free energy force field with a Lamarckian Genetic Algorithm, providing fast prediction of bound conformations with predicted free energies of association. AutoDock 4 is the new version that incorporates explicit conformational modelling of specified sidechains in the receptor. This property is an effective method for analysis of covalently attached ligands. AutoDock uses a grid based method to allow rapid evaluation of the binding

energy of trial conformations. In this method, the target protein is embedded in a grid. After that the ligand is sequentially placed at each grid point and the interaction energy between the ligand molecule and the target is computed, and the value is stored in the grid. The "Lamarckian" aspect is an added feature that allows individual conformations to search their local conformational space, finding local minima, and then pass this information to later generations (Morris *et al.*, 2009).

2.8.5 Post docking analysis

Reading a docking log is the first step in analysing the results of docking experiments. The docked conformations are sorted based on energy, from lowest to highest level. The best docked result can be considered to be the conformation with the lowest (docked) energy. At the end of each docking run, AutoDock gives an output of conformation with the lowest energy of the ligand it found during that run. This docked conformation consists of a position, orientation, and set of torsion angles, if any, and is characterized by an estimated free energy of binding, which is the sum of the intermolecular energy, the internal energy, and the torsional energy minus the unbound system's internal energy. AutoDock also reports van der Waals energy and an electrostatic energy for each atom (Morris *et al.*, 2008). The use of AutoDock to select the high ranking top compounds for further analysis was reviewed by Morris *et al.*

2.9 SELECTED PLANTS FOR *IN SILICO* SCREENING

It is well acknowledged that natural products particularly plant derived secondary compounds are the best potential source of medicine and such compounds generally induce no side effects or less side effects than the man made synthetic drugs (Lahlou, 2013). Five fruit crops have been selected to validate its activity against cobra venom. Selected fruit crops are common in Kerala and are reported to have activity against venom.

2.9.1 *Punica granatum* L.

Pomegranate is a plant that belongs to family Punicaceae, which is locally called as Anar, a fruit of great antiquity (Sangeetha and Jayaprakash, 2015). Its native place is from the Himalayas in northern India to Iran but has been cultivated and naturalized over the entire Mediterranean region since ancient times. Pomegranate is also cultivated in India and arid regions of Southeast Asia, the East Indies, and tropical Africa (Albrecht *et al.*, 2004). In Ayurvedic medicine the pomegranate is considered "a pharmacy unto itself" and is used as an antiparasitic agent, (Aviram and Dornfeld, 2001) a "blood tonic", (Batra *et al.*, 1968) and to heal aphthae, diarrhea, and ulcers (Batta and Rangaswami, 1973). Several studies are going on based on pomegranate to determine its action on prevention and treatment of cancer, erectile dysfunction, cardiovascular disease, diabetes, dental conditions, and skin allergy. Investigations were also carried out to determine antioxidant, anticarcinogenic and antiinflammatory properties of pomegranate constituents (Ahmed *et al.*, 2013 and Singh *et al.*, 2002). Whole part of Pomegranate plant has been used against snake bite traditionally and it is made into paste and applied on the bitten spot (Ntume and Anywar, 2015).

2.9.2 *Syzygium cumini* L.

The genus Syzygium is one among the genera of the myrtle family Myrtaceae which is native to the tropics, particularly to tropical America and Australia (Ayyanar and Babu, 2012). *Syzygium cumini* is a common traditional medicinal plant, whose parts have been pharmacologically proven to possess hypoglycemic, antibacterial and anti-HIV activities (Kusumoto *et al.*, 1995; Bhuiyan *et al.*, 1996; Ravi *et al.*, 2004). Different parts of plant, such as bark, leaves, fruit and seeds have been used in various traditional systems of medicine (Teixeira *et al.*, 1997). The leaves are used to treat leucorrhoea, stomachache, fever, dermopathy, (Warrier *et al.*, 1996) constipation, inhibit blood discharges in the feaces (Bhandary *et al.*, 1995) and reduce radiation induced DNA damage (Jagetia and Baliga, 2002). Fruits of this plants are used in Siddha, Ayurveda, Unani besides other folklore system of medicine in India as

stomachic astringent, antiscorbutic, diuretic, antidiabetic, enlargement of spleen and chronic diarrhea (Migliato, 2005). Jamun fruit is an effective food remedy for correcting liver disorders. In India, bark paste of the plant is used as an ethno medicine against snakebite in Kalahandi district of Orissa (Nayak *et al.*, 2004).

2.9.3 *Mangifera indica* L.

Mangifera indica L. belong to family Anacardiaceae, the origins of this fruit tree is from the East of India and Birmane. There are over 500 varieties in the world (Nathalie *et al.*, 2007). The genus Mangifera consisting of numerous species of tropical fruiting tree in the flowering plant family Anacardiaceae. It is commonly known as Mangoes and is one of the most famous and important fruit worldwide. The plant is widely grown and cultivated among tropical regions. The mango is indigenous to the Indian subcontinent, Southeast Asia and Africa (Fowomola *et al.*, 2010). Mango is the most widely exploited fruit for food, juice, flavor and fragrance and is a common ingredient in new functional foods often called super fruits (Kittiphoom *et al.*, 2012). Antioxidants and enzymes present in the mango fruits are believed to play an important role in the prevention and in the protection of cancer and heart disease (Orijajogun *et al.*, 2014). As a protective measure against snakebite, inflorescence of the plant is massaged on hands and applied on bitten part by the Tharu tribe of Devipatan division in the Terai belt of Uttar Pradesh (Kumar *et al.*, 2006).

2.9.4 *Tamarindus indica* L.

Tamarindus indica, belongs to the family Fabaceae, commonly known as Tamarind tree, is one of the fruit tree species that is used as traditional medicine (Kuru, 2014). Every part of plant (root, body, fruit, and leaves) has rich nutritional value and also have broad usage in the area of medicine. According to World Health Organization report, tamarind fruit is an ideal source of all essential amino acids except tryptophan (Glew *et al.*, 2005). The seed of tamarind has also becomes an important, accessible protein source especially in countries where protein malnutrition is a common problem. Tamarind is also effective against the treatment of diarrhoea and as

a laxative. The hepatoprotective activity of leaves are proven since it has poly-hydroxylated compounds, with many of them flavonolic in nature (Escalona *et al.*, 1995; Meléndez and Carriles, 2006). Along with the seeds, bark also have medicinal properties. Due to their antimicrobial, antifungal and antiseptic effects; tamarind leaves have an extensive ethnobotanical use (EscalonaArranz *et al.*, 2010; Lans, 2007). The aboriginals of Jalgaon district, Maharastra, India uses the seed poeder to treat snakebite, spoonful powder with honey is consumed thrice a day after every two hours (Pawar and Patil, 2007).

2.9.5 *Phyllanthus emblica* L.

Phyllanthus emblica Linn is a monoecious glabrous or pubescent deciduous tree that belongs to the family Euphorbiaceae (Rehman *et al.*, 2007). Indian gooseberry or amla is the common name of *Phyllanthus Emblica* and is one among the most important medicinal plant in the Indian traditional system, mainly in Ayurveda. To treat a range of diseases, various parts of the plant are used. The fruit can be used either alone or in combination with other plants to treat many ailments such as common cold and fever; as a diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, alterative, antipyretic, anti-inflammatory, hair tonic; to prevent peptic ulcer and dyspepsia, and as a digestive (Baliga and Dsouza, 2011). Amla is an important constituent of triphala, which is an Ayurvedic herbal formula that helps to cleanse the mucus of digestive villi. It is also reported that the gallic acid, which is a major polyphenol, present in amla suppresses the growth of cancer cells (Kaur *et al.*, 2005). Antisnake venom activity of *Phyllanthus emblica* and *Vitex negundo* were explored for the first time against *Naja kaouthia* and *Vipera russellii* venom. Both of the venom was antagonized by the plant extracts in both in vivo and in vitro studies. *Vipera russellii* venom induced coagulant, haemorrhage defibrinogenating and inflammatory activities were significantly neutralized by both plant extracts (Alam and Gomes, 2003). The tribes of Nasik district, Maharastra, India, uses stem infusion orally as an antidote (Patil and Patil, 2005).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled "*In silico* evaluation of anti-cobra venom activity in selected fruit crops." was done at JNTBGRI, Puthenthope and College of Agriculture, Vellayani.

3.1 PREPARATION OF TARGET PROTEINS.

The fourteen toxic proteins present in the venom of *Naja naja* L. (Indian Cobra), were selected as the target molecules. They are Phospholipase A2, Long neurotoxin 1, Long neurotoxin 2, Long neurotoxin 3, Long neurotoxin 4, Long neurotoxin 5, Acetylcholinesterase, L-aminoacid oxidase, Cobramine A, Cobramine B, Cytotoxin 3, Cobrotoxin, Serine protease and Proteolase. The three dimensional (3D) structures of Phospholipase A2 (PDB ID 1A3D) and Cobrotoxin (PDB ID 1COD) were procured from RCSB Protein Data Bank. The 3D structures of nine proteins *viz.*, Cobramine A (Swissprot ID P01447), Cobramine B (Swissprot ID P01440), Cytotoxin 3 (Swissprot ID P24780), Long neurotoxin 1 (Swissprot ID P25668), Long neurotoxin 2 (Swissprot ID P25669), Long neurotoxin 3 (Swissprot ID P25671), Long neurotoxin 4 (Swissprot ID P25672), Long neurotoxin 5 (Swissprot ID P25673) and Proteolase (Swissprot ID Q9PVK7) were retrieved from SWISSMODEL repository. Three Cobra venom proteins were modelled as per homology modelling using SWISSMODEL workspace. The primary sequence of serine protease was retrieved from Swissprot (ID: P86545) and submitted the same sequences on online Basic Local Alignment Search Tool for protein (BLASTp) on NCBI website. The primary sequence data of L-aminoacid oxidase and acetylcholinesterase of *Naja naja* (Indian Cobra) were not available in protein databases. However, the sequence of L-aminoacid oxidase in *Naja naja atra* (Chinese Cobra) (Swissprot ID A8QL58) and acetylcholinesterase in *Naja naja oxiana* (Central Asian Cobra) (Swissprot ID Q7LZG1), which are close relatives of *N. naja* were available in Swissprot database. Hence using these sequences, the templates such as 1EA5 for acetylcholinesterase and 1REO for L-aminoacid oxidase were selected through BLASTp analysis and the 3D structures were created in

SWISSMODEL and used in place of *N. naja* L-aminoacid oxidase and Acetylcholinesterase respectively.

3.2 SELECTION OF PLANT SPECIES AND PREPARATION OF LIGANDS

Based on the literature survey, easy availability and its wide usage as snake antidote by the traditional healers, especially throughout in Kerala State of India, five plant species *viz.*, *Punica granatum* (L.), *Syzygium cumini* (L.) *Mangifera indica* (L.), *Tamarindus indica* (L.) and *Phyllanthus emblica* (L.) were selected. The details regarding chemical structure of molecules present in these plants were searched in various literature and open access databases like Dr. Duke's phytochemical and Ethnobotanical Database (http://phytochem.nal.usda.gov/), PubChem (https://pubchem.ncbi.nlm.nih.gov/), FooDB (http://www.foodb.ca/) and ChemSpider (http://www.chemspider.com). Phytochemicals having molecular weight not greater than 1000 Da were selected for docking.

The chemical structure of compounds that are not available in open access databases were created using the software application ChemSketch and the 3D structures were generated in online tool named CORINA (https://www.mn-am.com/online_demos/corina_demo). Accordingly, 147 phytochemicals reported in *Punica granatum* were selected for docking. Similarly, 95 phytochemicals from *Syzygium cumini* were retrieved from databases. Likewise, in *Mangifera indica*, 99 molecules retrieved from databases were selected for docking and structure of 1 phytomolecule was created. In the same way, structures of 78 phytomolecules from *Tamarindus indica* were found in databases and structure of 2 phytochemicals were drawn using the tool. Consequently, in the case of *Phyllanthus emblica*, 94 phytochemicals were recovered from databases.

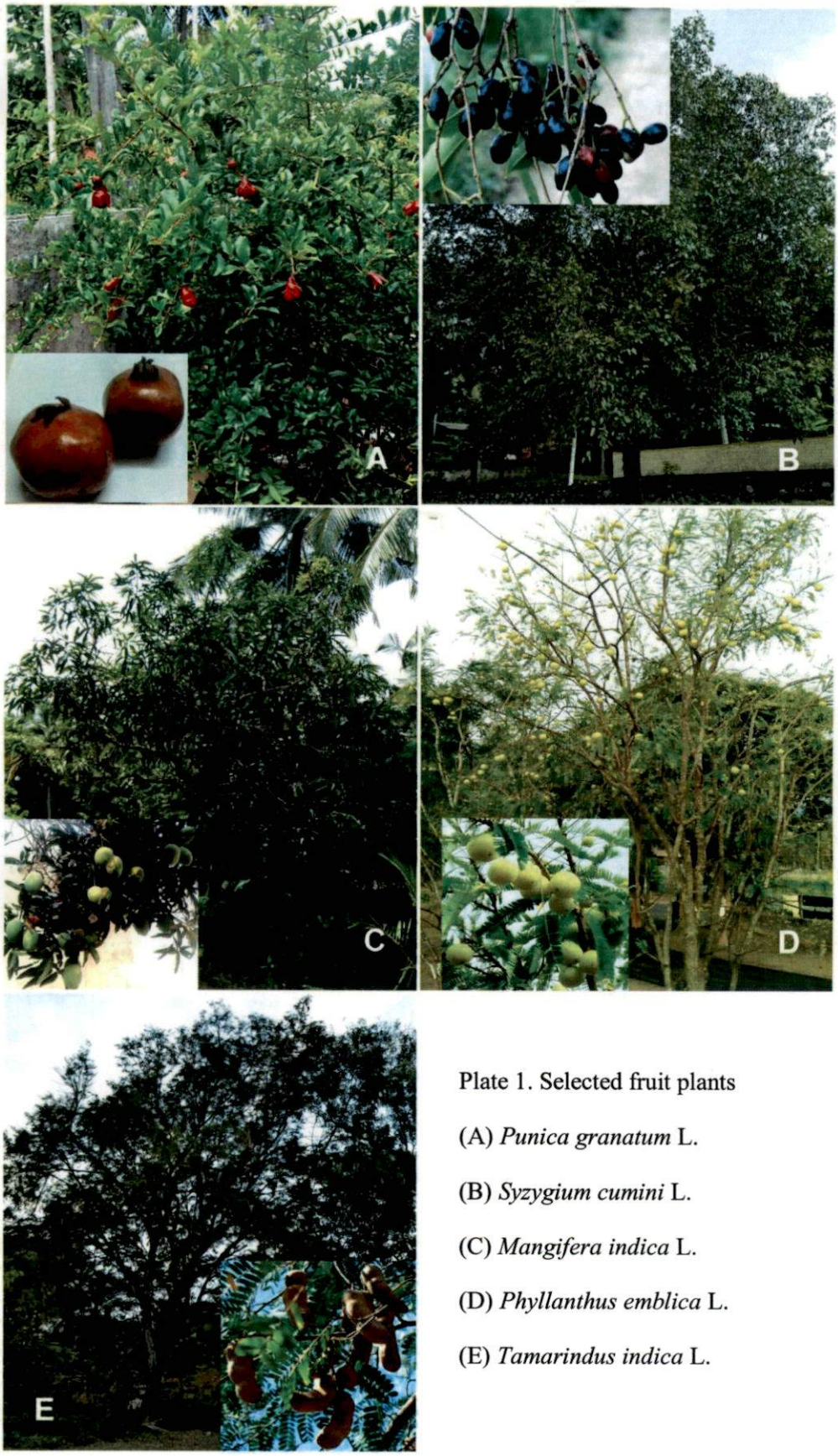


Plate 1. Selected fruit plants

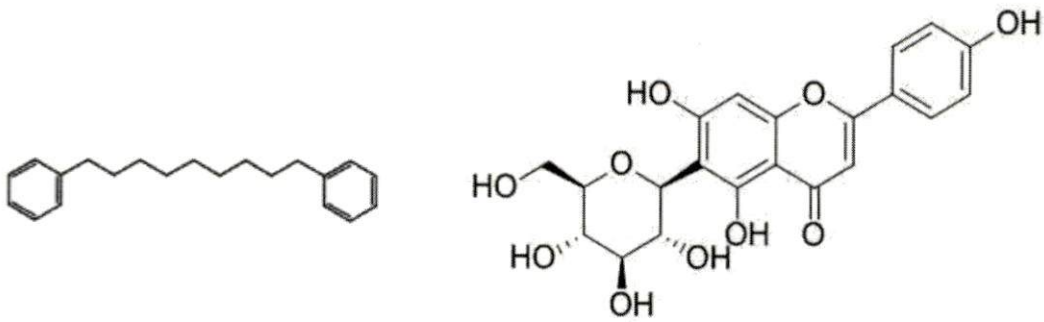
(A) *Punica granatum* L.

(B) *Syzygium cumini* L.

(C) *Mangifera indica* L.

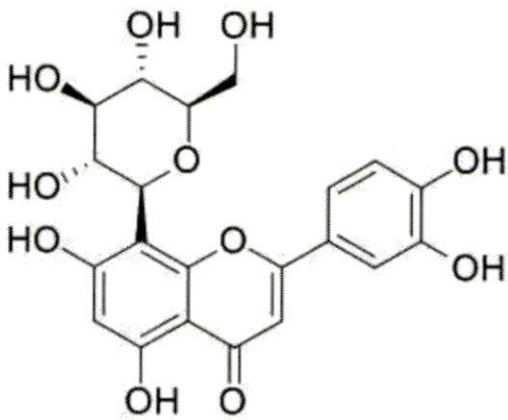
(D) *Phyllanthus emblica* L.

(E) *Tamarindus indica* L.



A

B



C

Figure 1.Structure of phytochemicals drawn using ChemSketch tool (A) 1,9 - Diphenyl nonane (B) Isovitexin (C) Orientin

3.3 DOCKING

3.3.1 AutoDock

The selected phytochemicals from all the five plants were docked into the binding site of each of the cobra venom proteins using open access software application tool named AutoDock 4.2 following the standard procedure (Morris *et al.*, 2008). The grid was centered on the active site of each target protein and grid spacing was set at 0.375 Å with 40×40×40 dimension. The ligand bound to the protein complex were further analyzed for its binding affinity. The top ranked molecule with free energy of binding ≤ -5 kcal/mol were selected and were further analyzed to find the lead molecule using PyMol. URL: http://autodock.scripps.edu/downloads/autodock registration/ autodock-4-2-download-page/

3.3.2 PatchDock

Docking was done using PatchDock following the standard procedure (Duhovny *et al.*, 2005). The proteins and ligands were uploaded on the online PatchDock server and the results were received through e-mail. The result page contained top ranked 20 docked solutions. PatchDock is available at http:// bioinfo3d. cs.tau.ac.il/PatchDock/.

3.3.3 HexServer

The docking tool, HexServer was downloaded from the URL: http://hex.loria.fr/ and installed and docking was performed. The HexServer provides fast and convenient way to generate high quality docking results. The docking result page was downloaded as compressed format and value was recorded.

3.3.4 iGEMDOCK

The tool provides an interactive interphase to prepare the protein binding site and ligand library. Each protein was uploaded and binding site was defined using the

option 'prepare binding site' and ligands were also uploaded in the PDB format. Each compound in the library was docked individually and the result page contained interactions such as energy, Van der Waal's and electrostatic. URL: http://gemdock.life.nctu.edu.tw/dock/download.php

3.4 FINDING LEAD MOLECULE THROUGH DST METHOD

To reduce the error in selection of leads, the molecules that showed best hits in Autodock were subjected to further docking using iGEMDOCK, Patchdock and HEX Server. The scores obtained were statistically analyzed following Dempster-Shafer Theory and identified best leads. The docked results in Autodock 4.2, iGEMDOCK, Patchdock and HEX Server were documented in a .xls spread sheet file format and uploaded on the website http://allamapparao.org/dst/ application tool to get the DST result.

3.5 TOOLS FOR VISUALIZING THE DOCKED RESULTS

After the docking is completed the docked results are visualized with the aid of following tools.

3.5.1 PyMol

PyMol produces high quality 3D structures of protein bound to the ligand molecule. The result file in the PDB format is uploaded to the PyMol and the 3D structure can be visualized. The software also provides the information about the number of hydrogen bond, the residue to which the bond is formed and type of bond. The image of the particular protein can be saved in the .jpg format.

3.5.2 LigPlot

LigPlot software was used to visualize the 2D structure of the protein and ligand interaction. The PDB file was uploaded and the RUN button was clicked. The 2D structure of ligand bound to the protein residue was obtained as result. The Hydrogen bond formed between protein and ligand along with its bond length was also depicted. Hydrophobic interaction of protein residue with ligand were also obtained.

RESULTS

4. RESULTS

4.1 DOCKED RESULT OF *PUNICA GRANATUM* L.

A total of 147 phytochemicals present in *Punica granatum* were docked with each of the 14 selected cobra venom target molecules by using AutoDock 4.2. The docked structures having free energy of binding ≤ -5 kcal/mol were selected as the hit molecules. Lead molecules were selected on the basis of parameters such as free energy of binding, inhibition constant (Ki) and number of Hydrogen bonds. Of the 147 phytochemicals derived from *P. granatum* 31 compounds did not show inhibitory activity on any of the 14 cobra venom proteins. Of the 147 phytochemicals screened, maximum number of hit molecules were obtained against the major toxic protein, phospholipase A2 (99) followed by L aminoacid oxidase (87), proteolase (50), cobrotoxin (23), acetyl choline esterase (20), long neurotoxin 4 (19), long neurotoxin 5 (19), long neurotoxin 3 (9), long neurotoxin 1 (7), serine protease (7), cobramine B (4), long neurotoxin 2 (1), and no hit molecule was obtained against cobramine A.

Of the 99 hit molecules identified against phospholipase A2, 9 compounds having least free energy of binding were selected as lead molecules. The selected lead molecules with least free energy of binding on the order of merit are gamma sitosterol (-11.52 kcal/mol), lupenone (-10.41 kcal/mol), beta sitosterol (-9.99 kcal/mol), betulic acid (-9.83 kcal/mol), cycloartenol acetate (-9.43 kcal/mol), ursolic acid (-9.34 kcal/mol), friedelin (-9.24 kcal/mol), friedooleanan-3-one (-9.23 kcal/mol) and Oleanolic acid (-9.03 kcal/mol). Among the selected nine lead molecules the compound gamma sitosterol have least free energy of binding, hydrophobic interaction with critical residue Asp48 and one hydrogen bond with Asn52 and therefore considered as the best lead. Twenty three compounds have showed inhibitory activity on cobrotoxin, out of these, eight compounds having least binding energy and inhibition constant such beta sitosterol (-6.08 kcal/mol), cycloartenol acetate (-6.03 kcal/mol), friedelin (-6.94 kcal/mol), friedooleanan-3-one (-6.77 kcal/mol), gamma

sitosterol (-6.35 kcal/mol), lupenone (-7.20 kcal/mol), oleanolic acid (-6.41 kcal/mol) and stigmasterol (-6.77 kcal/mol). Lupenone was selected as the best lead molecule since it have least binding energy value of (-7.20 kcal/mol) and inhibition constant is 5.32 μM. Lupenone also showed 2 hydrogen bond with Thr37 and Arg28. It also interacts with critical residue Arg33 through hydrophobic interaction.

Seven phytochemicals have inhibitory effect on long Neurotoxin 1, among these four compounds such as serotonin (-6.27 kcal/mol), para menth-1-en-4-ol (-5.54 kcal/mol), cymene (-5.44 kcal/mol) and sedridine (-5.49 kcal/mol) were selected as the lead molecules and para menth-1-en-4-ol was selected as the best lead molecule since it have two hydrogen bonds and hydrophobic interaction with critical residue Thr22. Only one phytochemical *viz.* 4n-propylresorcinol showed inhibitory effect on long neurotoxin 2 and its binding energy was -5.25 kcal/mol and the compound showed three hydrogen bonds. Nine hit molecules were obtained against long neurotoxin 3 and four compounds were selected as lead molecule based on low binding energy and inhibition constant. The lead molecules were serotonin (-6.60 kcal/mol), p-menth-1-en-4-ol (-5.53 kcal/mol), 4n-propylresorcinol (-5.52 kcal/mol) and sedridine (-5.50 kcal/mol). The compound 4n-propylresorcinol was selected as the best lead molecule since it has three Hydrogen bonds with target protein and also showed hydrophobic interaction with the critical residue Tyr21. Nineteen phytochemicals showed inhibitory activity on long neurotoxin 4 and five lead molecule such as stigmasterol (-6.10 kcal/mol), oxandrolone (-6.10 kcal/mol), esterone (-5.95 kcal/mol), estriol (-5.62 kcal/mol) and ursolic acid (-5.62 kcal/mol) were selected as lead molecules. Among these, estriol was selected as the best lead molecule since it has three hydrogen bonds with target protein and hydrophobic interaction with critical residue Gln55. Nineteen phytochemicals showed inhibitory activity on long neurotoxin 5 and five compounds *viz.* beta sitosterol (-5.87 kcal/mol), ethyl brevifolin carboxylate (-5.62 kcal/mol), serotonin (-5.62 kcal/mol), ursolic acid (-5.62 kcal/mol) and stigmasterol (-5.50 kcal/mol) were selected as lead molecules. The compound ethyl brevifolin carboxylate

was selected as the best lead molecule since it has two hydrogen bonds with target protein and hydrophobic interaction with the critical residue Arg33.

Only four compounds showed inhibitory activity on cobramine B. In the order of merit tartaric acid (-6.38 kcal/mol) showed least binding energy followed by oxalic acid (-5.93 kcal/mol), succinic acid (-5.57 kcal/mol) and o-coumarinic acid (-5.02 kcal/mol respectively. Tartaric acid has been selected as the lead molecule since its binding energy and inhibition constant (21.03 μM) was least, established nine hydrogen bond with target protein and hydrophobic interaction with critical residue Lys23. Sixteen phytochemicals showed binding energy ≤ -5 kcal/mol with the target cytotoxin 3 and five compounds having top least binding energy such as ursolic acid (-6.87 kcal/mol), cycloartenol acetate (-6.74 kcal/mol), friedooleanan-3-one (-6.55 kcal/mol), beta sitosterol (-6.09 kcal/mol) and stigmasterol (-5.89 kcal/mol) were selected as the lead molecules. The difference in binding energy among the compounds was negligible and beta sitosterol was selected as the best lead molecule since it have hydrophobic interaction with critical residue Lys18 and two hydrogen bond with Arg36, Leu6.

Proteolase was inhibited by 50 phytochemicals and six among them were selected as the lead molecules. The selected lead molecules were flavogallol (-6.72 kcal/mol), cycloartenol acetate (-6.35 kcal/mol), friedelin (-6.20 kcal/mol), beta-sitosterol (-6.17 kcal/mol), lupenone (-6.15 kcal/mol) and stigmasterol (-6.12 kcal/mol). The binding energy differences between the selected lead molecules were negligible and the molecule cycloartenol acetate was selected as the best lead molecule since has one hydrogen bond with critical residue Asp 477. Twenty phytochemicals showed inhibitory activity on acetyl choline esterase among these, five compounds *viz.* beta sitosterol (-6.93 kcal/mol), lupenone (-6.25 kcal/mol), cycloartenol acetate (-6.09 kcal/mol), 17- alpha estradiol (-6.04 kcal/mol) and 17-beta estradiol (-6.04 kcal/mol) were selected as the lead molecules. Among the lead molecules beta sitosterol was selected as the best lead molecule since it have ΔG_{bind} value of -6.93 kcal/mol, inhibition constant 8.35 μM and form one hydrogen bond with Asn181. Beta sitosterol

also showed hydrophobic interaction with critical residue Ser76. Eighty seven phytochemicals showed binding energy less than -5 kcal/mol with the target, L aminoacid oxidase and 13 phytochemicals having binding energy ≤ -7.0 kcal/mol were selected as lead molecules. The selected lead molecules were betulinic acid (10.62 kcal/mol), lupenone (-10.51 kcal/mol), ursolic acid (-10.12 kcal/mol), friedooleanan-3-one (-10.11 kcal/mol), 17-Alpha estradiol (-9.78 kcal/mol), cycloartenol acetate (-9.58 kcal/mol), gamma-sitosterol (-9.57 kcal/mol), friedelin (-9.38 kcal/mol), estradiol (-9.34 kcal/mol), maslinic acid (-9.11 kcal/mol), stigmasterol (-9.04 kcal/mol), beta-sitosterol (-8.68 kcal/mol) and gallagic acid (-7.09 kcal/mol). Among the lead molecules ursolic acid was selected as the best lead compound since it have binding energy of -10.12 kcal/mol, inhibition constant of 38.34 μM and three hydrogen bonds. Ursolic acid also interacts with critical residue Arg95. Against serine protease, seven phytochemicals have binding energy ≤ -5 kcal/mol and among these four molecules such oxandrolone (-6.07 kcal/mol), lupenone (-5.94 kcal/mol), beta sitosterol (-5.82 kcal/mol) and oxalic acid (-5.58 kcal/mol) were selected as lead molecules. Oxandrolone was selected as the best lead molecule since it has three hydrogen bonds with target protein and among these one hydrogen bond was formed with critical residue Ser31.

Table 2. Docked result of *Punica granatum*.

Target	Lead molecule	BE (kcal/ mol)	KI (μM)	H B	Bond type	Bond length
PLA2	Beta sitosterol	-9.99	0.047	0		
	Betulic acid	-9.83	0.061	2	Tyr63 OHO Asn52 OHO	2.77, 2.78
	Cycloartenol acetate	-9.43	0.122	0		
	Friedelin	-9.24	0.169	0		
	Friedooleanan-3-one	-9.23	0.170	0		
	Gamma sitosterol	-11.5	0.003	1	Asn52 NHO	3.01
	Lupenone	-10.4	0.023	0		
	Oleanolic acid	-9.03	0.241	2	Gly29 OHO , Cys92 OHO	2.88, 2.59
	Ursolic acid	-9.34	0.141	1	Cys92 OHO	2.71
CBT	Beta sitosterol	-6.08	34.78	1	Arg28 NHO	2.97
	Cycloartenol acetate	-6.03	38.29	1	Thr37 NHO	2.68
	Friedelin	-6.94	8.12	0		
	Friedooleanan-3-one	-6.77	10.94	0		
	Gamma sitosterol	-6.35	22.24	1	Thr37 OHO	2.5
	Lupenone	-7.20	5.32	2	Thr37 OHO, Arg28 NHO	2.83, 2.44
	Oleanolic acid	-6.41	19.87	1	Arg28 NHO	2.88
	Stigmasterol	-6.77	10.98	0		
LN 1	Para Menth-1-en-4-ol	-5.54	87.36	2	Pro64 OHO Cys62 OHO	2.60, 3.17
	Cymene	-5.44	103.1	0		
	Sedridine	-5.49	94.24	2	Pro71 OHO, Gln55 NHO	3.01, 2.97
LN 2	4n-Propyl resorcinol	-5.25	142.26	3	Met62 NHO Gly88 NHO Tyr389 OHO	2.8, 1.8, 2.7
LN 3	Para Menth-1-en-4-ol	-5.53	88.53	1	Gln55 NHO	2.73
	4n-Propyl resorcinol	-5.52	90.39	3	Arg70 OHO, Pro71 OHO, Gln55 NHO	2.8, 3.2, 1.9
	Sedridine	-5.50	92.69	3	Cys62 NHO, Gln55 OHO, Pro71 OHO	2.1, 2.1, 2.9
LN 4	Esterone	-5.90	46.95	2	Pro64 OHO, Thr22 OHO	2.74, 2.6
	Estriol	-5.62	75.90	3	Pro66 OHO, OHO, OHO	3.0, 2.8, 2.9
	Oxandrolone	-6.10	33.87	2	Phe645 OHO, Pro64 OHO	2.73, 2.70
	Stigmasterol	-6.10	33.55	1	Arg36 NHO	3.21
	Ursolic acid	-5.59	79.48	2	Val37 OHO Arg36 NHO	2.61, 3.02
LN 5	Beta sitosterol	-5.87	50.16	1	Thr67 OHO	2.70
	Ethyl brevifolin carboxylate	-5.62	75.55	2	Val37 NHO Asp27 OHO	2.93, 2.86
	Stigmasterol	-5.50	93.50	0		
	Ursolic acid	-5.62	76.35	0		
CA	-	-	-	-	-	-

Table 2. Continued

CB	O-coumarinic-acid	-5.02	208.1	4	Cys38 NHO, OHO Lys12 NHO, Arg36 OHO	3.02, 2.93, 2.74, 3.00
	Oxalic acid	-5.93	45.00	5	Lys18 NHO Cys38 OHO, OHO Lys12 NHO, NHO	2.64, 2.55, 2.96, 2.49, 2.82
	Succinic acid	-5.57	83.32	5	Lys18 NHO Cys38 OHO, NHO Lys12 NHO NHO	2.76, 2.80, 2.88, 2.73, 3.20
	Tartaric acid	-6.38	21.03	9	Lys18 OHO Cys38 OHO, OHO, OHO, OHO Lys12 NHO Arg36 OHO Tyr22 OHO, OHO	2.92, 2.68, 2.72, 3.19, 2.57, 2.56, 3.10, 2.55, 3.25
CYT3	Beta sitosterol	-6.09	34.56	2	Arg36 OHO Leu6 NHO	2.50, 3.32
	Cycloartenol acetate	-6.74	11.44	2	Asp40 NHO Val41 NHO	3.11, 3.17
	Friedooleanan-3-one	-6.55	15.93	0		
	Stigmasterol	-5.89	48.49	1	Tyr22 OHO	2.69
	Ursolic acid	-6.87	9.20	1	Tyr22 OHO	2.62
PRT	Beta sitosterol	-6.17	30.22	8		
	Flavogallol	-6.72	11.83	1	Lys458 NHO	2.77
	Cycloartenol acetate	-6.35	22.07	1	Asp477 NHO	2.85
	Friedelin	-6.20	28.32	0		
	Lupenone	-6.15	30.79	0		
	Stigmasterol	-6.12	32.69	1	Lys458 NHO	2.76
ACE	17-Alpha estradiol	-6.04	37.63	1	Ser96 OHO	2.81
	17-Beta estradiol	-6.04	37.36	1	Ser96 OHO	2.86
	Beta sitosterol	-6.93	8.35	1	Asn181 OHO	2.87
	Cycloartenol acetate	-6.09	34.55	1	Gly78 NHO	3.01
	Lupenone	-6.25	26.29	0		
LAAO	17-Alpha estradiol	-9.78	0.067	5	Met108 NHO, OHO, NHO Ser445 OHO	2.9, 3.03, 2.9, 2.73
	Beta sitosterol	-8.68	0.437	0		
	Betulic acid	-10.6	0.016	5	Arg109 NHO, OHO, NHO Met104 OHO Ser445 OHO Gly444 OHO	3.08, 2.74, 3.32, 3.05, 3.08
	Cycloartenol acetate	-9.58	0.095	1	Arg90 NHO	3.05
	Estradiol	-9.34	0.141	3	Met108 NHO, OHO Arg109 NHO	3.00, 3.32, 3.07
	Friedelin	-9.38	0.132	1	Ala63 NHO	3.12
	Friedooleanan-3-one	-10.1	0.038	1	Ala63 NHO	2.87
	Gallagic acid	-7.09	0.006	2	Gly64 NHO Ala63 NHO	2.96, 2.91

Table 2. Continued

	Gamma sitosterol	-9.57	0.097	2	Glu82 OHO Ser84 NHO	2.52, 2.87
	Lupenone	-10.5	0.019	1	Lys343 NHO	3.0
	Maslinic acid	-9.11	0.211	3	Met62 NHO Gly88 NHO Tyr389 OHO	3.2, 2.96, 2.73
	Stigmasterol	-9.04	0.023	0		
	Ursolic acid	-10.1	0.038	3	Gly88 OHO Arg90 NHO Met62 NHO	3.25, 2.59, 3.23
SP	Beta sitosterol	-5.82	53.94	2	Leu40 OHO, NHO	2.65, 2.71
	Lupenone	-5.94	43.96	1	Lys46 NHO	3.09
	Oxalic acid	-5.58	81.09	1	Lys46 NHO	2.57
	Oxandrolone	-6.07	35.23	3	Ser31 OHO Gln45 NHO Lys46 NHO	2.86, 3.05, 3.14
	Succinic acid	-6.08	34.73	4	Leu40 OHO, NHO	2.65, 2.71

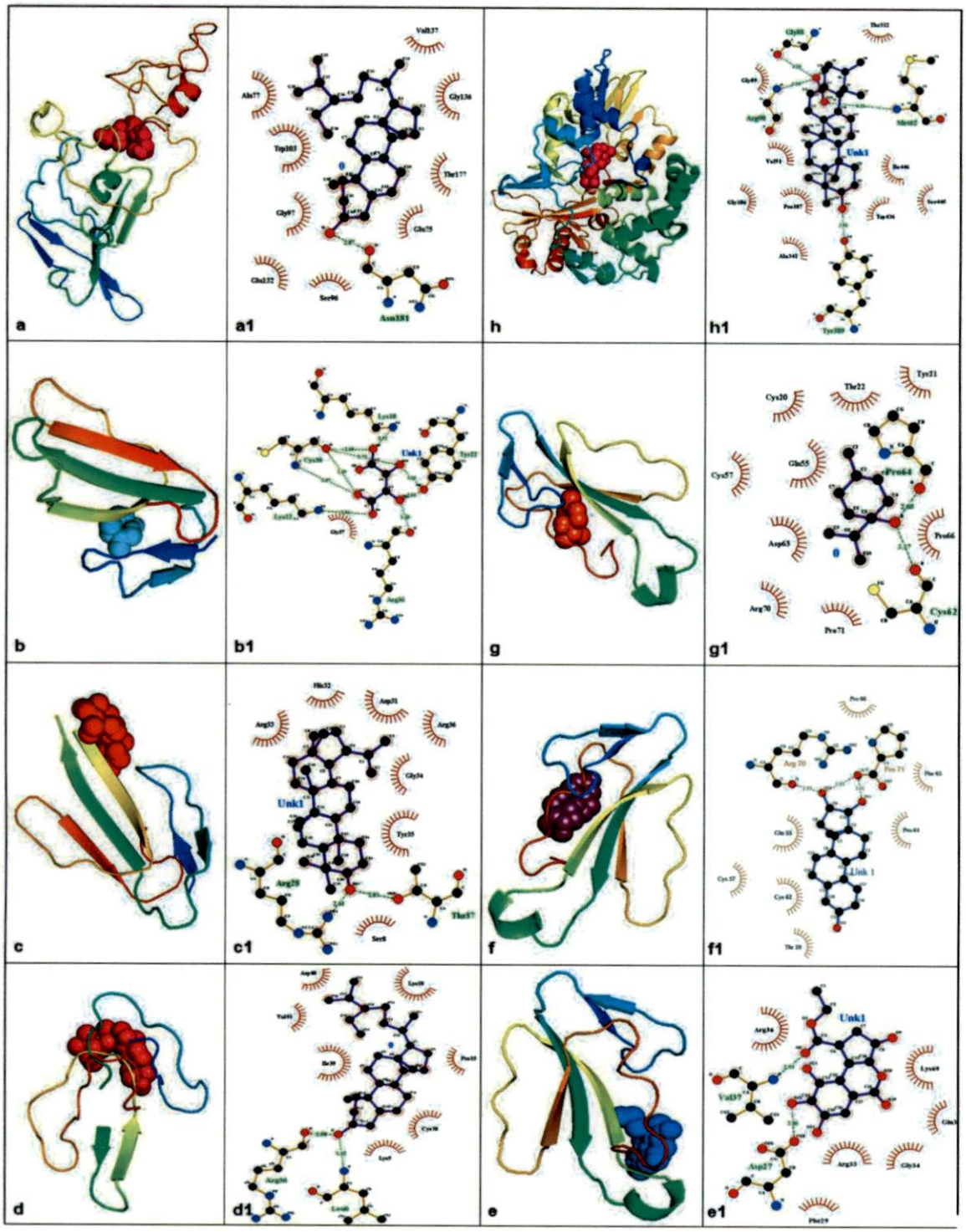


Figure 4. Docked structures of cobra venom proteins and lead molecule from *Punica granatum* in PyMol and LigPlot respectively: (a and a1) ACE and Beta-sitosterol (b and b1) CB and Tartaric acid (c and c1) CBT and Lupenone (d and d1) CYT3 and Beta-sitosterol (e and e1) LN5 and Ethyl brevifolin (f and f1) LN4 and Estriol (g and g1) LN1 and P- menth-1-en-4-ol (h and h1) LAAO and Ursolic acid

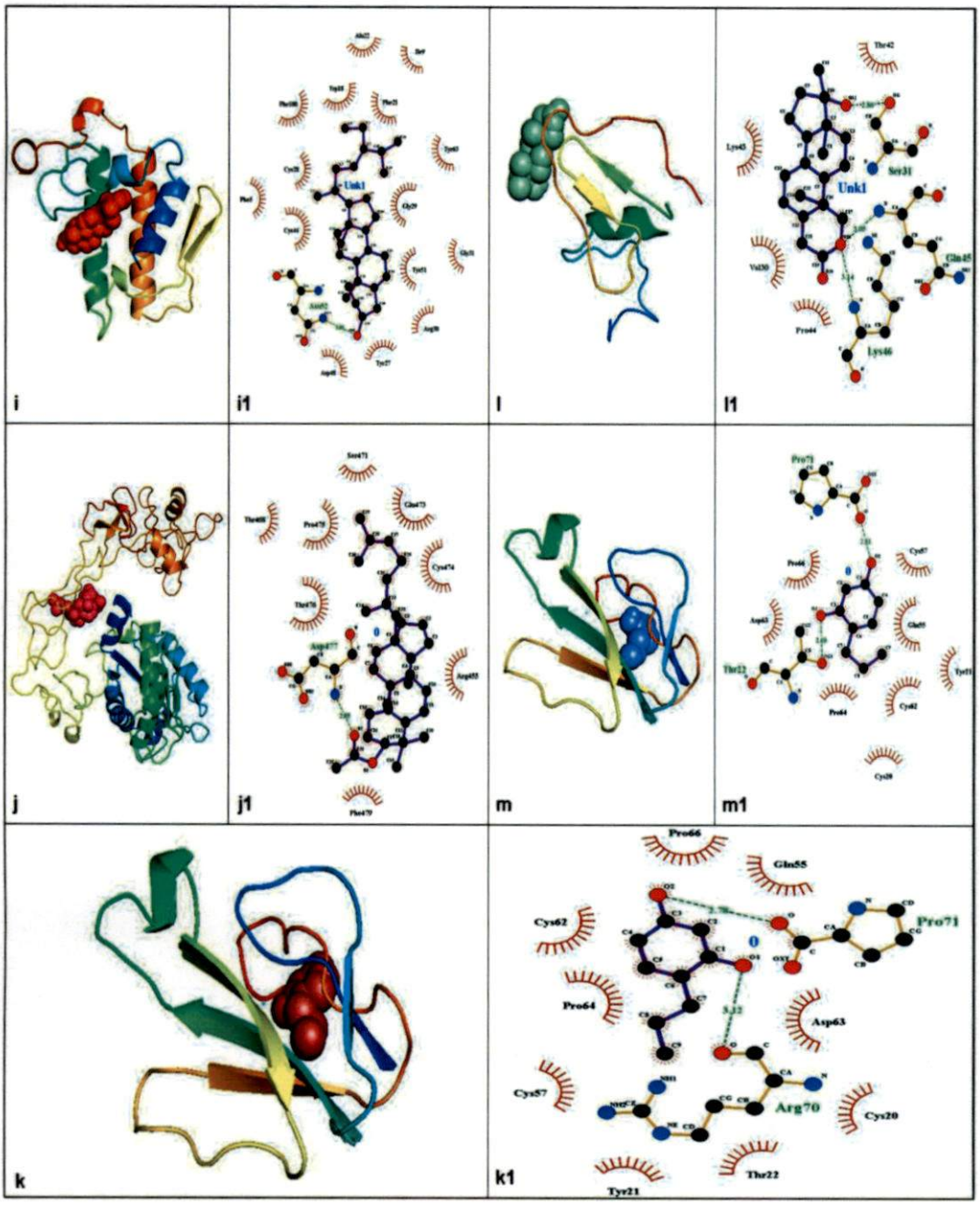


Figure 4 Continued. Docked structures of cobra venom proteins and lead molecule from *Punica granatum* in PyMol and LigPlot respectively: (i and i1) PLA2 and Gamma-sitosterol (j and j1) PRT and Cycloartenol (k and k1) LN3 and 4n-Propylresorcinol (l and l1) SP and Oxandrolone (m and m1) LN2 and 4n-Propylresorcinol

4.2 DOCKED RESULT OF *SYZYGIUM CUMINI* L.

A total of 95 phytochemicals from *Syzygium cumini* were docked with each of the 14 selected cobra venom protein and the results indicated that the plant contains phytochemicals for inhibiting venom proteins except long neurotoxin 2. Out 95 phytochemicals 81 hit molecules obtained against phospholipase A2 and the number of hit molecules obtained against other targets in the order of merit were long neurotoxin 3 (15), proteolase (15), cytotoxin 3 (10), Long neurotoxin 5 (9), cobrotoxin (8), cobramine B (7), L-aminoacid oxidase (7), acetylcholineesterase(5), Long neurotoxin 1 (5), Long neurotoxin 4 (4), serine protease (3) and cobramine A (1). The details of inhibitory activity of phytochemicals are as follows.

A total of 81 compounds showed binding energy less than -5 kcal/mol with phospholipase A2 , of these, five molecules having least binding energy such as epi-friedlanol (-9.08 kcal/mol), friedelin (-9.08 kcal/mol), oleanolic acid (-8.05 kcal/mol), Terpinyl valerate (-8.09 kcal/mol) and Malvidin - 3- glucoside (-7.86 kcal/mol) were selected as lead molecules. The compound epi-friedlanol was selected as the best lead since it has hydrogen bond and hydrophobic interaction with Asp 48. Eight hit molecules were obtained against cobrotoxin. Of these, five compounds *viz.* friedelin (-6.94 kcal/mol, epifriedelanol (-6.54 kcal/mol), epi-friedlanol (6.54 kcal/mol), friedelanol (6.52 kcal/mol),) and oleanolic acid (-6.41 kcal/mol) were selected as the lead molecules. Among these, except friedelin all others showed one hydrogen bond with the target protein and the binding energy among the lead compounds were negligible The compound epifriedelanol was selected as the best lead since it has comparatively less bond length and hydrophobic interaction with Arg33 .

Five compounds have inhibitory activity on long neurotoxin 1. They are dihydrocarvyl acetate (-5.68 kcal/mol), alpha terpineol (-5.76 kcal/mol) beta selinene (-5.65 kcal/mol), beta terpinene (-5.56 kcal/mol) and alpha santalol (-5.33 kcal/mol). Among these, alpha santalol was selected as the best lead molecule since it has three

hydrogen bonds with residues Gln55, Cys57 and Cys20. Fifteen phytochemicals showed inhibitory activity on long neurotoxin 3, among these, five compounds such as dihydrocarvyl acetate (-5.67 kcal/mol), geranyl butyrate (-5.57 kcal/mol), alpha terpineol (5.79 kcal/mol), beta selinene (-5.76 kcal/mol) and beta terpinene (-5.55 kcal/mol). The compound alpha terpineol was selected as the best lead molecule since it forms one hydrogen bond with Pro64 and also hydrophobicaly interact with Tyr21, which is the critical residue. Four phytochemicals showed inhibitory activity on long neurotoxin 4. They were cyanidin (-5.21 kcal/mol), delfinidin (-5.46 kcal/mol), eucarvone (-5.10 kcal/mol) and myricetin (-5.03 kcal/mol). The compound cyanidin was selected as the best lead since it forms two hydrogen bonds with Thr22 and Cys5. Nine compounds showed inhibitory activity on long neurotoxin 5 and five compounds such as alpha cadinol (-5.31 kcal/mol), muurolol (-5.31 kcal/mol), oleanolic acid (-5.94 kcal/mol), alpha copaene (-5.63 kcal/mol) and alpha santalol (-5.29 kcal/mol) were selected as lead molecules. Among these, alpha santalol was selected as the best lead molecule since it forms three hydrogen bond with Gly34 and hydrophobic interaction with Asp27 and critical residue Arg33.

Only one compound, calacorene have inhibitory activity on cobramine A. This compound showed moderate binding energy of -5.15 kcal/mol, inhibition constant of 168.18 μM and no hydrogen bond was present. Seven compounds showed inhibitory activity on cobramine B, of these five molecules such as alpha cadinol (-5.80 kcal/mol), muurolol (-5.80 kcal/mol), calacorene (-5.47 kcal/mol), citic acid (-5.43 kcal/mol) and gamma cadinene (-5.24 kcal/mol) were selected as lead molecules. The compound citic acid was selected as the best lead since it has six hydrogen bonds with the target protein at residues Lys12, Cys38, Tyr22 and Arg36. Ten compounds showed inhibitory effect on cytotoxin 3. Of these, five compounds *viz.* friedelin (-5.82 kcal/mol), friedelanol (-5.71 kcal/mol), epifriedelanol (-5.37 kcal/mol), betulinic acid (-5.21 kcal/mol), and alpha cadinol (-5.28 kcal/mol) were selected as the lead molecules. Among these, the

compound alpha cadinol was selected as the best lead since it has two hydrogen bonds with residues Arg36 and Leu6. Other compounds did not show any hydrogen bond.

Fifteen compounds have inhibitory activity on proteolase and the selected lead molecules 3, 3'-di–o–methyl ellagic acid (-5.99 kcal/mol), acetyl oleanolic acid (-6.04 kcal/mol), friedelin (-6.20 kcal/mol), isoquercetin (-5.96 kcal/mol) and myricetin 3-o-4-acetyl-L rhamnopyranoside (-6.05 kcal/mol). The compound 3, 3'-di–o–methyl ellagic acid was selected as the best lead since it forms three hydrogen bond with Cys474 and the critical residue Asp477. Five phytochemicals showed inhibitory activity on acetylcholine esterase, of these, betulinic acid was selected as the best lead since it showed least binding energy and three hydrogen bonds with the residues Glu75 and Phe36. Hydrogen bond was not present in other hit molecules. Seven compounds showed inhibitory activity on L aminoacid oxidase. Among these, the selected lead molecules were betulinic acid (-9.66 kcal/mol), oleanolic acid (-9.54 kcal/mol), epifriedelanol (-9.52 kcal/mol), friedelanol (-9.51 kcal/mol) and acetyl oleanolic acid (-9.25 kcal/mol). The compound betulinic acid was selected as the best lead molecule since it forms hydrogen bonds with Thr447and Lys 343. Only 3 compounds such as acetyl oleanolic acid (-5.17 kcal/mol), betulinic acid (-5.51 kcal/mol) and epi-friedlanol (-5.64 kcal/mol) showed binding energy less than -5 kcal/mol against serine protease. Of these, betulinic acid was selected as the best lead since it forms a hydrogen bond with Lys34 and hydrophobic interaction with critical residue Ser31.

Table 3. Docked result of *Syzygium cumini*.

Target	Lead molecule	BE (kcal/ mol)	KI (μM)	H B	Bond type	Bond length
PLA2	Epi-friedlanol	-9.08	0.22	1	Gly31 OHO	3.32
	Friedelin	-9.08	0.221	0		
	Oleanolic acid	-8.05	1.26	1	Tyr63 OHO	2.73
	Terpinyl valerate	-8.09	1.17	1	Gly29 NHO	2.88
	Malvidin- 3-glucoside	-7.86	3.1	0		
CBT	Epifriedelanol	-6.54	16.03	1	Ser8 OHO	2.87
	Epi-friedlanol	-6.54	16.09	1	Ser8 OHO	2.89
	Friedelanol	-6.52	16.60	1	Ser8 OHO	2.92
	Friedelin	-6.94	8.12	0		
	Oleanolic acid	-6.41	19.87	1	Arg28 NHO	2.88
LN 1	Dihydrocarvyl acetate	-5.68	68.18	1	Gln55 NHO	2.77
	santalol	-5.33	124.0	3	Gln55 OHOCys57 SHO Cys20 OHO	2.69, 3.19, 2.98
	Alpha terpineol	-5.76	59.78	1	Pro64 OHO	2.43
	Beta selinene	-5.65	72.24	0		
	Beta terpinene	-5.56	83.69	0		
LN 2	-	-	-	-	-	-
LN 3	Dihydrocarvyl acetate	-5.67	69.52	1	Gln55 NHO	
	Geranyl butyrate	-5.57	83.21	1	Arg70 NHO	2.76
	Alpha terpineol	-5.79	56.69	1	Pro64 OHO	3.27
	Beta selinene	-5.76	59.64	0		2.44
	Beta terpinene	-5.55	85.96	0		
LN 4	Cyanidin	-5.21	150.9	2	Thr22 OHOCys56 OHO	2.68, 2.86
	Delfinidin	-5.46	99.59	0		
	Eucarvone	-5.10	182.7	1	Thr22 OHO	2.9
	Myricetin	-5.03	206.5	0		
LN 5	Alpha cadinol	-5.31	127.7	1	Lys69 NHO	2.92
	Muurolol	-5.31	128.2	1	Lys69 NHO	2.92
	Oleanolic acid	-5.94	44.25	0		
	Alpha copaene	-5.63	74.26	0		
	Alpha santalol	-5.29	131.8	3	Gly34 OHOAsp27 NHO, OHO	2.70, 3.08, 2.46
CA	Calacorene	-5.15	168.1	0		
CB	Calacorene	-5.47	98.08	0		
	Citic acid	-5.53	88.24	6	Lys12 NHO,NHO Cys38 NHO Tyr22 OHO,OHO Arg36 OHO	2.89, 3.20, 2.89, 2.77, 2.90, 2.76
	Muurolol	-5.80	56.30	1	Pro30 OHO	2.54
	Alpha cadinol	-5.80	56.30	1	Pro30 OHO	2.5
	G-cadinene	-5.24	144.5	0		

Table 3. Continued

CYT3	Betulinic acid	-5.21	151.6	0		
	Epifriedelanol	-5.37	115.5	0		
	Friedelanol	-5.71	65.73	0		
	Friedelin	-5.82	54.59	0		
	Alpha cadinol	-5.28	135.0	2	Arg36 OHO Leu6 NHO	2.61, 2.90
PRT	3,3'di-O-methyl ellagic acid	-5.99	40.98	3	Cys474 NHO, OHOAsp477 OHO	3.23, 2.53, 2.82
	Acetyl oleanolic acid	-6.04	37.68	1	Cys474 OHO	3.24
	Friedelin	-6.20	28.32	0		
	Isoquercetin	-5.96	43.05	7	Glu473 OHO, OHOCys474 NHO Arg455 NHO, NHO Glu440 NHO Glu439 OHO	2.62, 2.9, 2.7, 2.78, 3.9, 2.9, 2.5
	Myricetin 3-O-4-acetyl-L rhamnopyranoside	-6.05	36.85	6	Glu473 OHO, OHOArg455 NHO, NHO, NHO Cys474 NHO	2.81, 2.67, 2.98, 2.62, 2.79, 3.31
ACE	Betulinic acid	-5.35	119.5	3	Glu75 NHO, OHOPhe36 OHO	3.11, 2.94, 2.66
	Epifriedelanol	-5.85	51.53	0		
	Epi-friedlanol	-5.85	51.15	0		
	Friedelanol	-5.66	71.01	0		
	Friedelin	-6.56	15.63	0		
LAAO	Acetyl oleanolic acid	-9.25	0.165	1	Tyr389 OHO	3.31
	Betulinic acid	-9.66	0.082	3	Thr447 OHO, NHO Lys 343 NHO	2.78, 3.27, 2.92
	Epifriedelanol	-9.52	0.105	0		
	Epi-friedlanol	-9.52	0.105	0		
	Friedelanol	-9.51	0.106	0		
	Oleanolic acid	-9.54	0.101	3	Gly88 OHO Met62 NHO Tyr389 OHO	2.99, 3.20, 2.50
SP	Acetyl oleanolic acid	-5.17	162.13	0		
	Betulinic acid	-5.51	92.01	1	Lys34 NHO	2.98
	Epi-friedlanol	-5.64	73.38	0		

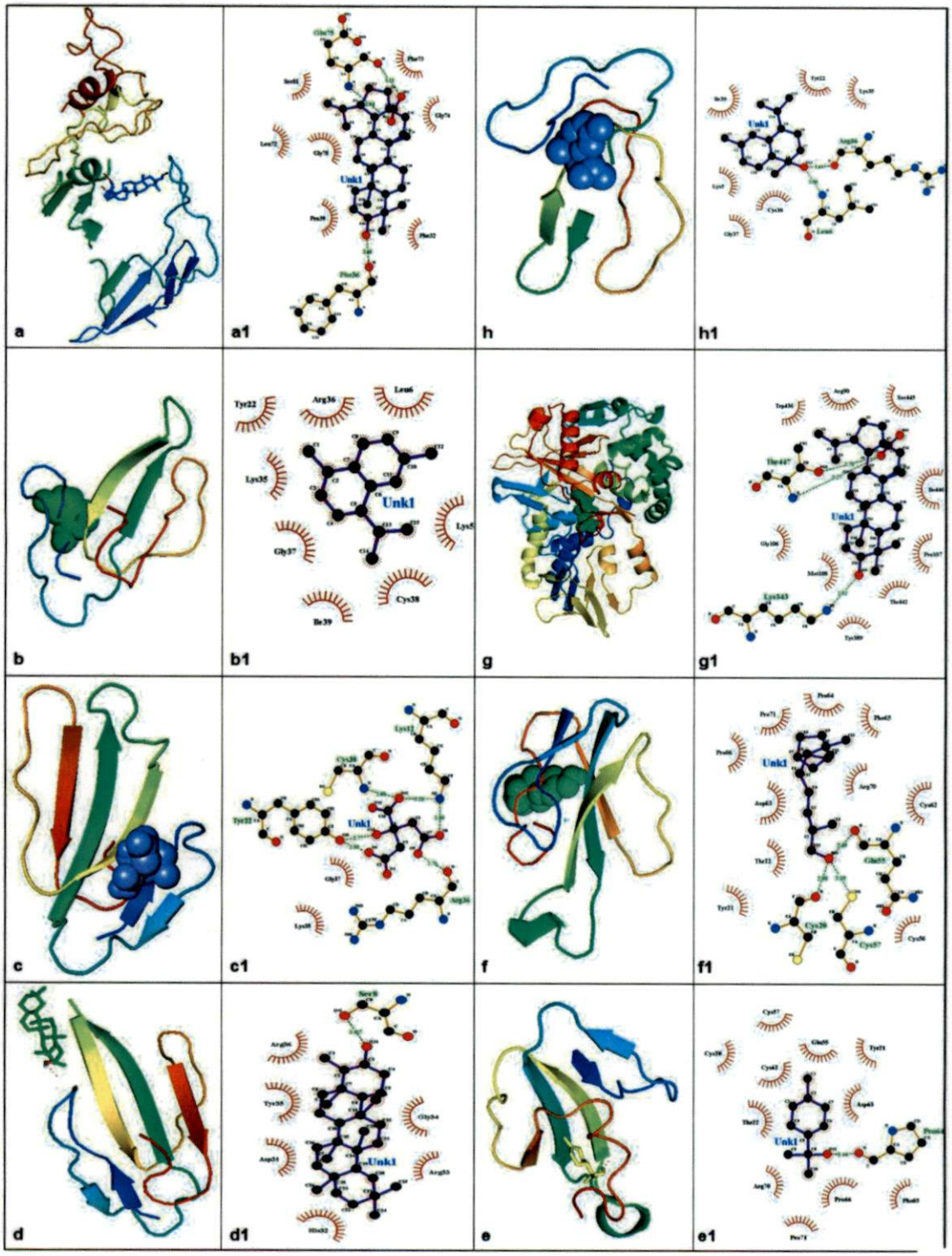


Figure 5. Docked structures of cobra venom proteins and lead molecule from *Syzygium cumini* in PyMol and LigPlot respectively: (a and a1) ACE and Betulinic acid (b and b1) CA and Calacorene (c and c1) CB and Citic acid (d and d1) CBT and Epifriedelanol (e and e1) LN3 and Alpha terpineol (f and f1) LN1 and Alpha santalol (g and g1) LAAO and Betulinic acid (h and h1) CYT3 and Alpha candinol

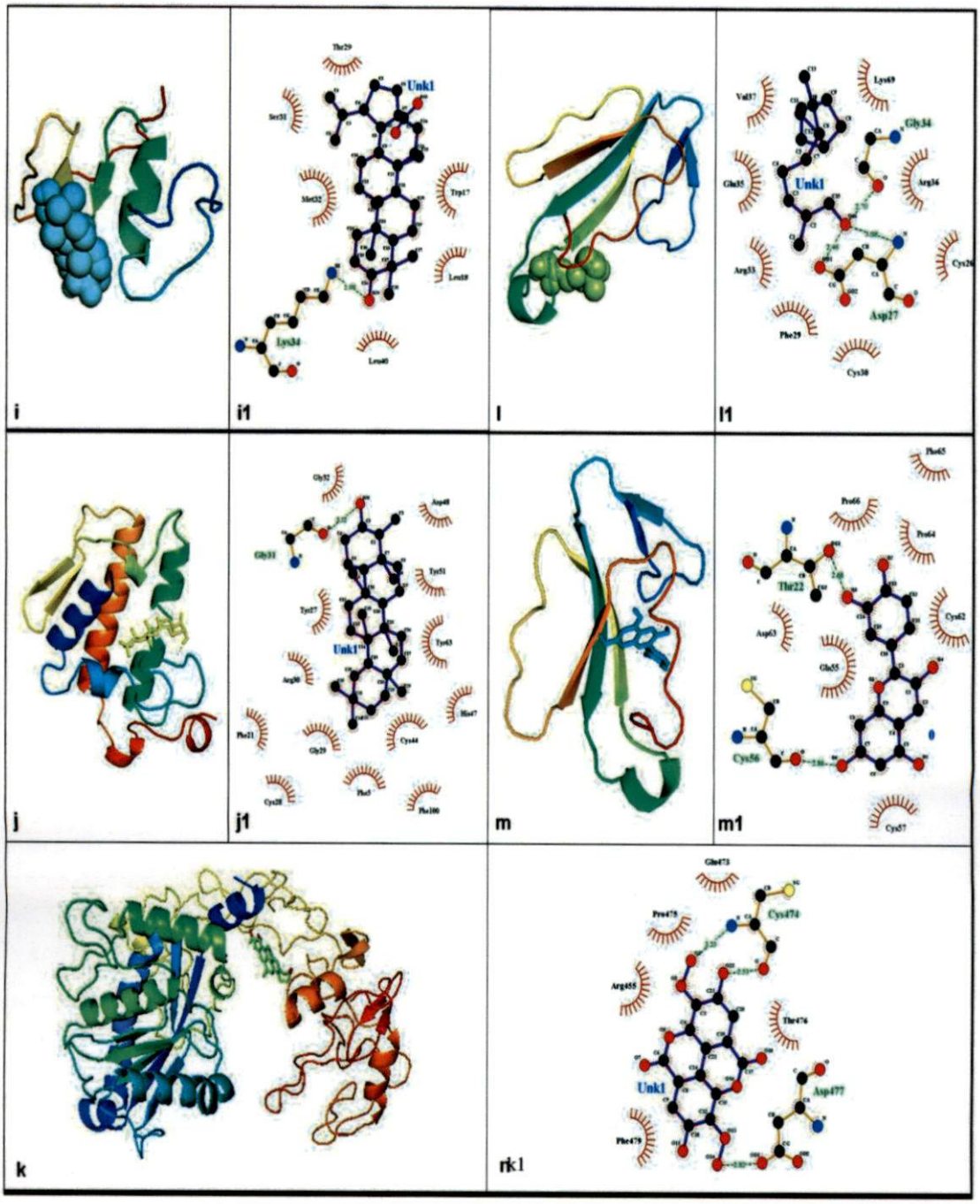


Figure 5 Continued. Docked structures of cobra venom proteins and lead molecule from *Syzygium cumini* in PyMol and LigPlot respectively: (i and i1) SP and Betulinic acid (j and j1) PLA2 and Epifriendlanol (k and k1) PRT and 3', 3'-di-O-Methyl ellagic acid (l and l1) LN5 and Alpha santalol (m and m1) LN4 and Cyanidin

4.3 DOCKED RESULT OF *MANGIFERA INDICA* L.

A total 100 phytochemicals derived from *Mangifera indica* were docked with each of the 14 target proteins and the results revealed that the plant can inhibit all toxic cobra venom proteins. Out of 100 phytochemicals 13 compounds did not show inhibitory activity on any of the 14 target proteins. The number of hit molecules obtained against each venom protein in the order of merit was phospholipase A2 (84), L-aminoacid oxidase (60), cobramine A (22), long neurotoxin 5 (20), long neurotoxin 3 (17), cytotoxin 3 (13), serine protease (13), long neurotoxin 1 (12), long neurotoxin 4 (11), cobramine B (10), proteolase (8), acetylcholineesterase (7), cobrotoxin (6), long neurotoxin 2 (1) respectively. The details of selected lead molecules and the best lead molecule against each target protein were as follows.

A total 84 hit molecules ($\Delta G_{bind} \leq$-5.00 kcal/.mol) were obtained against phospholipase A2. Among these, five molecules having top least binding energy such as epicatechin-3-o-gallate (-9.18 kcal/mol), kaempferol-3-o-glucoside (-8.15 kcal/mol), quercetin 3 arabinoside (-8.16 kcal/mol), quercetin (-7.17 kcal/mol) and metadigallic acid (-7.07 kcal/mol) were selected as lead molecules. Among these, quercetin 3 arabinoside quercetin was selected as the best lead since it has two hydrogen bonds and one of the hydrogen bond established with the active residue His47. Six hit molecules such as isomangiferolic acid (-6.64 kcal/mol), mangiferonic acid (-6.58 kcal/mol), ambonic acid (-6.42 kcal/mol), ambolic acid (-6.39 kcal/mol), mangiferolic acid (6.11 kcal/mol) and Guaiol (-5.32 kcal/mol) were obtained against cobrotoxin. Among these, ambonic acid was selected as the best lead since it has three hydrogen bonds and hydrophobic interaction with the critical residue Arg33. Twelve hit molecules were obtained against long neurotoxin 1 and the five selected lead molecules were valencene (-6.20 kcal/mol), 7-epi-a-selinene (-6.06 kcal/mol), eremophyllene (-5.78 kcal/mol), g-gurjunene (-5.65 kcal/mol) and hinesol (-5.71 kcal/mol). Among these, only hinesol showed hydrogen bond with the target residue Pro71. Guaiol is the only hit molecules noted against long neurotoxin 2. The binding

energy and inhibition constant of guaiol was -5.27 kcal/mol and 136.22 uM respectively. It has a hydrogen bond with Thr47 and hydrophobic interaction with the critical residue Lys23. Seventeen hit molecules were noted against long neurotoxin 3 and the lead molecule were as same as in long neurotoxin 1. The compound hinesol was selected as the best lead. Eleven hit molecules were obtained against long neurotoxin 4. Among these, five molecules such as mangiferolic-acid (-5.87 kcal/mol), isomangiferolic-acid (-5.81 kcal/mol), 7-epi-a-selinene (-5.78 kcal/mol), eremophyllene (-5.58 kcal/mol) and guaiol (-5.59 kcal/mol), were selected as the lead. Among these, isomangiferolic-acid was selected as the best lead molecule since it has four hydrogen bonds with the residues Pro71, Glu55, Arg70 and Arg70. Of these, Glu55 was the critical residue. Twenty hit molecules were found against long neurotoxin 5. Of these, five compounds *viz.* ambolic acid (-6.45 kcal/mol), ambonic acid (-6.41 kcal/mol), guaiol (-6.26 kcal/mol), isomangiferolic-acid (-5.61 kcal/mol) and mangiferonic-acid (-7.53 kcal/mol) were selected as lead molecules. The compound ambolic acid was selected as the best lead since it has two hydrogen bonds with critical residue Arg33.

Twenty two hit molecules were obtained against cobramine A and the five selected lead molecules were 1-epi-cubenol (-5.70 kcal/mol), cyperene (-5.66 kcal/mol), beta chamigrene (-5.61 kcal/mol), pogostol (-5.51 kcal/mol) and guaiol (-5.29 kcal/mol). Among these, 1-epi-cubenol was selected as the best lead molecule since it has the least binding energy and two hydrogen bonds with Arg36 and Leu6 and also showed hydrophobic interaction with critical residue Tyr22. Ten phytochemicals showed inhibitory activity on cobramine B, of these, five compounds *viz.* alpha cadinol (-5.80 kcal/mol), 1-epi-cubenol (-5.56 kcal/mol), cyperene (-5.36 kcal/mol), allo aromadendrene (-5.30 kcal/mol) and guaiol (-5.17 kcal/mol) were selected as the lead molecules. The compound 1-epi-cubenol was selected as the lead molecule since it has two hydrogen bonds with Asn60 and Arg36. The compound also forms hydrophobic interaction with critical residue Lys23. Thirteen hit molecules were noted against

cytotoxin 3, of these, five molecules such as isomangiferolic acid (-7.66 kcal/mol), mangiferolic acid (-7.47 kcal/mol), caryophyllene oxide (-5.34 kcal/mol), alpha cadinol (-5.32 kcal/mol) and ambonic acid (-5.32 kcal/mol) were selected as lead molecules. The compound isomangiferolic acid was selected as the best lead since it has least binding energy (-7.66 kcal/mol) and inhibition four hydrogen bonds with the target molecule. One of the hydrogen bonds established with critical residue Lys18 and others were Val41, Arg36 and Leu6. Eight hit molecules were obtained against proteolase, of these, five compounds *viz.* ambonic acid (-5.89 kcal/mol), ambolic acid (-5.99 kcal/mol), isomangiferolic acid (-6.04 kcal/mol) mangiferolic acid (-6.24 kcal/mol) and mangiferonic acid (-6.57 kcal/mol) were selected as the lead molecules. The compound mangiferonic acid was selected as the best lead since it showed least binding energy and has two hydrogen bonds with Lys458 and Asn494. Seven hit molecules were noted against acetylcholine esterase. Among these, five molecules such as mangiferonic acid (-5.60 kcal/mol), mangiferolic acid (-5.52 kcal/mol), isomangiferolic acid (-5.40 kcal/mol), guaiol (-5.32 kcal/mol) and ambolic acid (-5.30 kcal/mol) were selected as lead molecules. The compound mangiferolic acid was selected as the best lead since it three hydrogen bond with the residues Glu75, Leu37 and Phe36 of the target protein and showed hydrophobic interaction with the critical residue Ser76. Sixty hit molecules were noted against L-aminoacid oxidase and the selected five lead molecules were isomangiferolic acid (-11.13 kcal/mol), mangiferolic acid (-11.08 kcal/mol), mangiferonic acid (-10.80 kcal/mol), ambolic acid (-10.76 kcal/mol), and ambonic acid (-9.75 kcal/mol). All the lead molecules have hydrogen bond with the active residue Arg90. Therefore, the compound isomangiferolic acid, which has least binding energy, was selected as the best lead. Thirteen hit molecules were obtained against serine protease and the selected lead molecules includes 4-o-methylgallic acid (-6.13 kcal/mol), ambonic acid (-6.54 kcal/mol), isomangiferolic-acid (-6.45 kcal/mol), mangiferonic acid (-6.86 kcal/mol) and para coumaric acid (-7.02 kcal/mol). The compound para coumaric acid having least binding energy and five hydrogen bonds was selected as the best lead.

Table 4. Docked result of *Mangifera indica.*

Target	Lead molecule	BE (kcal/mol)	KI (μM)	H B	Bond type	Bond length
PLA2	Epicatechin-3-O-gallate	-9.18	0.186	5	Arg30 NHO, OHO Cys92 OHO Asp93 OHO Trp18 OHO	2.58, 2.54, 3.26, 2.80, 3.15
	Kaempferol-3-O-glucoside	-8.15	1.06	9	Cys43 NHO, OHO, Cys44 OHO, Tyr27 OHO, Gly31 OHO, OHO, Gly29 NHO, OHO, Try18 OHO	2.65, 2.86, 2.79, 3.03, 2.91, 3.02, 3.25, 3.11, 2.83
	Quercetin	-7.17	5.59	2	Phe21 OHO Gly31 OHO	2.90, 2.80
	Metadigallic acid	-7.07	6.62	4	Trp18 OHO , Gly29 NHO, His47 NHOCys43 OHO	2.84, 2.97, 2.84, 2.78
	Quercetin 3 arabinoside	-8.16	1.04	2	His47 NHO, Trp18 OHO	2.55, 2.53
CBT	Ambolic acid	-6.39	20.82	3	Thr37 NHO, OHO Arg28 NHO	2.87, 2.83, 2.87
	Ambonic acid	-6.42	19.56	3	Thr37 NHO, Arg28 NHO His32 NHO	2.88, 2.70, 3.30
	Isomangiferolic acid	-6.64	13.56	2	Thr37 NHO, OHO	2.89, 2.63
	Mangiferolic acid	-6.11	33.04	1	Thr37 NHO	2.90
	Mangiferonic acid	-6.58	15.14	1	Tyr35 OHO	2.67
	Guaiol	-5.32	126.4			
LN 1	7-epi-a-selinene	-6.06	36.05	0		
	Eremophyllene	-5.78	57.75	0		
	Gamma gurjunene	-5.65	72.06	0		
	Hinesol	-5.71	65.28	1	Pro71 OHO	2.7
	Valencene	-6.20	28.49	0		
LN 2	Guaiol	-5.27	136.2	2	Lys23 NHOThr47 OHO	3.01, 3.11
LN 3	7-epi-a-selinene	-6.10	34.00	0		
	Eremophyllene	-5.78	58.12	0		
	Gamma gurjunene	-5.76	59.96	0		
	Hinesol	-5.80	56.24	1	Pro71 OHO	2.5
	Valencene	-6.26	25.60	0		
LN 4	7-epi-a-selinene	-5.79	56.62	0		
	Eremophyllene	-5.58	81.13	0		
	Guaiol	-5.59	79.51	1	Pro71 OHO	2.2
	Isomangiferolic acid	-5.81	55.25	4	Pro71 OHO , Glu55 OHO , Arg70 NHO, Arg70 NHO	1.8, 2.1, 2.2, 2.0
	Mangiferolic acid	-5.87	50.17	4	Pro71 OHO, OHO Cys56 NHO, OHO	2.5. 3.4, 1.7. 3.0
LN 5	Ambolic acid	-6.45	18.78	2	Arg33 NHO, NHO	2.87, 2.84
	Ambonic acid	-6.41	19.98	2	Arg33 OHO , Arg36 NHO	2.91, 2.78

Table 4. Continued

	Guaiol	-6.26	25.92	1	Asp27 OHO	2.74
	Isomangiferolica cid	-5.61	77.27	2	Arg36 NHO	2.1, 2.2
	Mangiferonic acid	-7.53	3.02	2	Val37 NHO, Arg36 NHO	2.89, 3.05
CA	1-epi-cubenol	-5.70	66.76	2	Arg36 OHO, Leu6 NHO	2.41, 2.56
	Beta chamigrene	-5.61	77.42	0		
	Cyperene	-5.66	71.06	0		
	Guaiol	-5.29	133.0	1	Cys38	2.94
	Pogostol	-5.51	90.95	1	Cys38 OHO	3.07
CB	Alpha cadinol	-5.80	56.48	1	Pro30 OHO	2.53
	1-epi-cubenol	-5.56	83.56	2	Asn60, Arg36 OHO, NHO	3.12, 2.95
	Cyperene	-5.36	117.1	0		
	Guaiol	-5.17	162.2	1	Pro30 OHO	2.68
	Allo aromadendrene	-5.30	131.3	0		
CYT3	Alpha cadinol	-5.32	126.4	2	Arg36 OHO, Leu6 NHO	2.57, 3.15
	Ambonic acid	-5.32	125.7	1	Val41 NHO	3.13
	Caryophyllene oxide	-5.34	121.3	1	Leu6 NHO	2.88
	Isomangiferolic acid	-7.66	2.43	4	Lys18 NHOVal41 NHOArg36 OHO, Leu6 NHO	2.92, 3.16, 2.50, 3.33
	Mangiferolic acid	-7.47	3.34	3	Lys18 NHOVal41 NHOArg36 OHO	2.93, 3.27, 2.54
PRT	Ambolic acid	-5.99	40.86	2	Lys458 NHO, Arg187 OHO	3.01, 2.76
	Ambonic acid	-5.89	48.41	2	Arg455 NHOGlu440 OHO	3.02, 3.14
	Isomangiferolic acid	-6.04	37.14	2	Lys458 NHO, Cys474 OHO	2.71, 2.64
	Mangiferolic acid	-6.24	26.85	2	Arg455 NHO, Lys458 NHO	2.77, 2.69
	Mangiferonic acid	-6.57	15.26	2	Lys458 NHOAsn494 OHO	2.99, 2.83
ACE	Guaiol	-5.32	126.6	2	Glu75 NHO, OHO	2.69, 3.05
	Isomangiferolic acid	-5.40	110.6	2	Glu75 OHO, Leu37 OHO	2.96, 2.73
	Mangiferolic acid	-5.52	89.39	3	Glu75 OHO, Leu37 OHO, Phe36 OHO	3.01, 3.12, 2.63
	Mangiferonic acid	-5.60	78.25	1	Glu75 OHO	2.80
	Ambolic acid	-5.30	130.2	4	Glu75 NHO, OHO, Phe36 OHO, Leu37 OHO	2.77, 3.01, 2.65,2.91
LAAO	Ambolic acid	-10.7	0.012	2	Lys343 NHO Arg109 NHOArg90	2.62, 3.11
	Ambonic acid	-9.75	0.070	2	Met108 NHO Met62 NHO Arg90	2.95, 2.79
	Isomangiferolic acid	-11.1	6.9	4	Arg109 NHO, NHO, OHO Met108 NHOArg90	2.85,3.12, 3.13, 2.97

Table 4. Continued

	Mangiferolic acid	-11.0	0.007	3	Arg109 NHO, NHO, Met108 NHOArg90	2.87, 3.16, 2.69
	Mangiferonic acid	-10.8	0.012	5	Arg109 NHO, NHO, OHO Met108 NHOMet62 NHOArg90	3.08, 2.96, 3.13, 2.66, 3.11
SP	4-O-Methylgallic acid	-6.13	32.01	4	Thr35 OHO , Asn33 OHO , Leu41 OHO , Lys46 NHO	2.94, 3.15, 2.89, 2.93,
	Ambonic acid	-6.54	16.17	1	His38 OHO	2.97
	Isomangiferolic acid	-6.45	18.59	2	Thr29 OHO Leu40 OHO	2.88, 2.86
	Mangiferonic acid	-6.86	9.43	1	His38 OHO	2.83
	Para coumaric acid	-7.02	7.17	5	Thr39 OHO Thr35 OHO NHO, Lys46 NHO Lys43 NHO	2.98, 3.23, 2.99, 2.77, 2.57

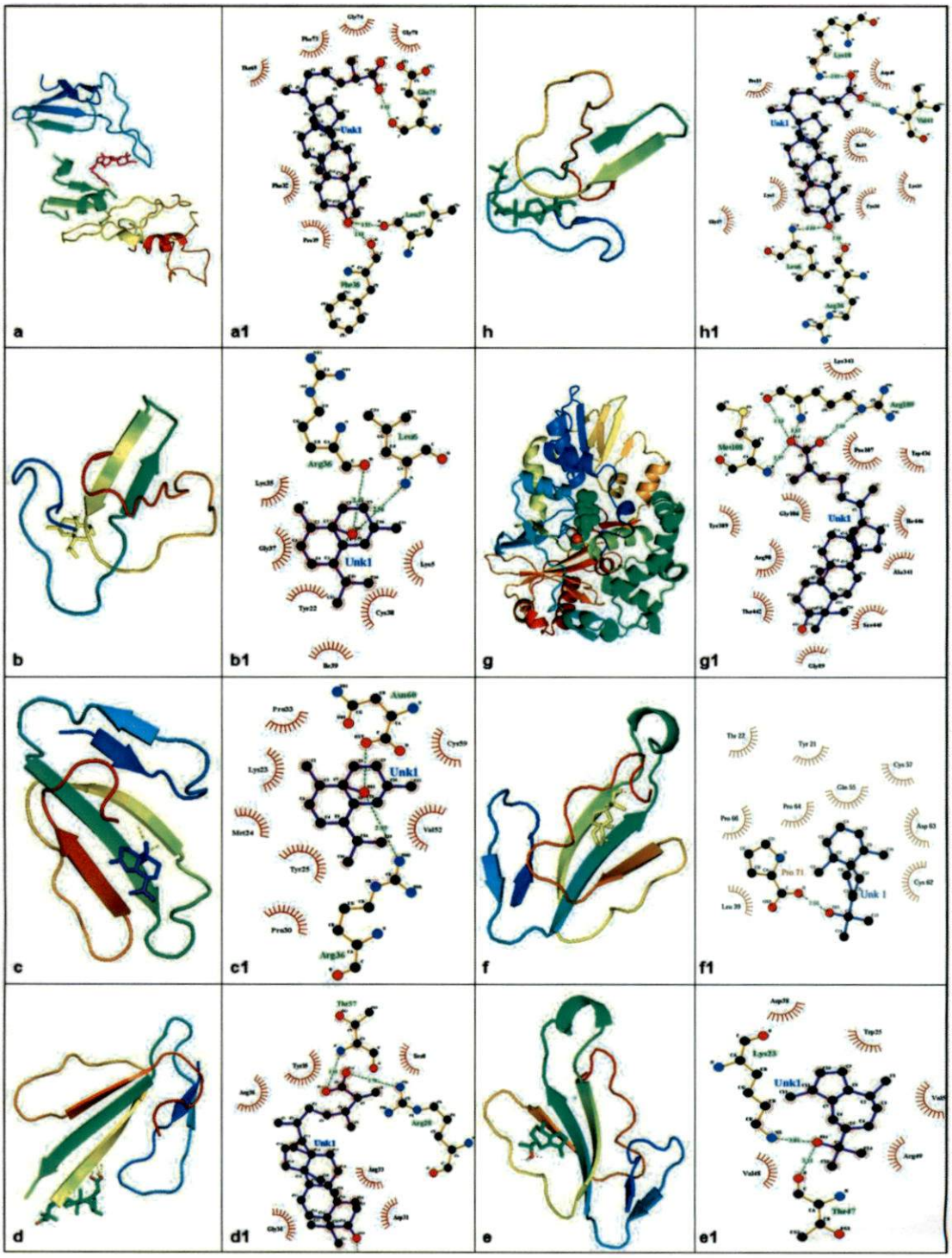


Figure 6. Docked structures of cobra venom proteins and lead molecule from *Mangifera indica* in PyMol and LigPlot respectively: (a and a1) ACE and Mangiferolic acid (b and b1) CA and 1-ep- cubenol (c and c1) CB and 1-epi-cubenol (d and d1) CBT and Ambonic acid (e and e1) LN2 and Guaiol (f and f1) LN1 and Hinesol (g and g1) LAAO and Isomangiferolic acid (h and h1) LN2 and Guaiol

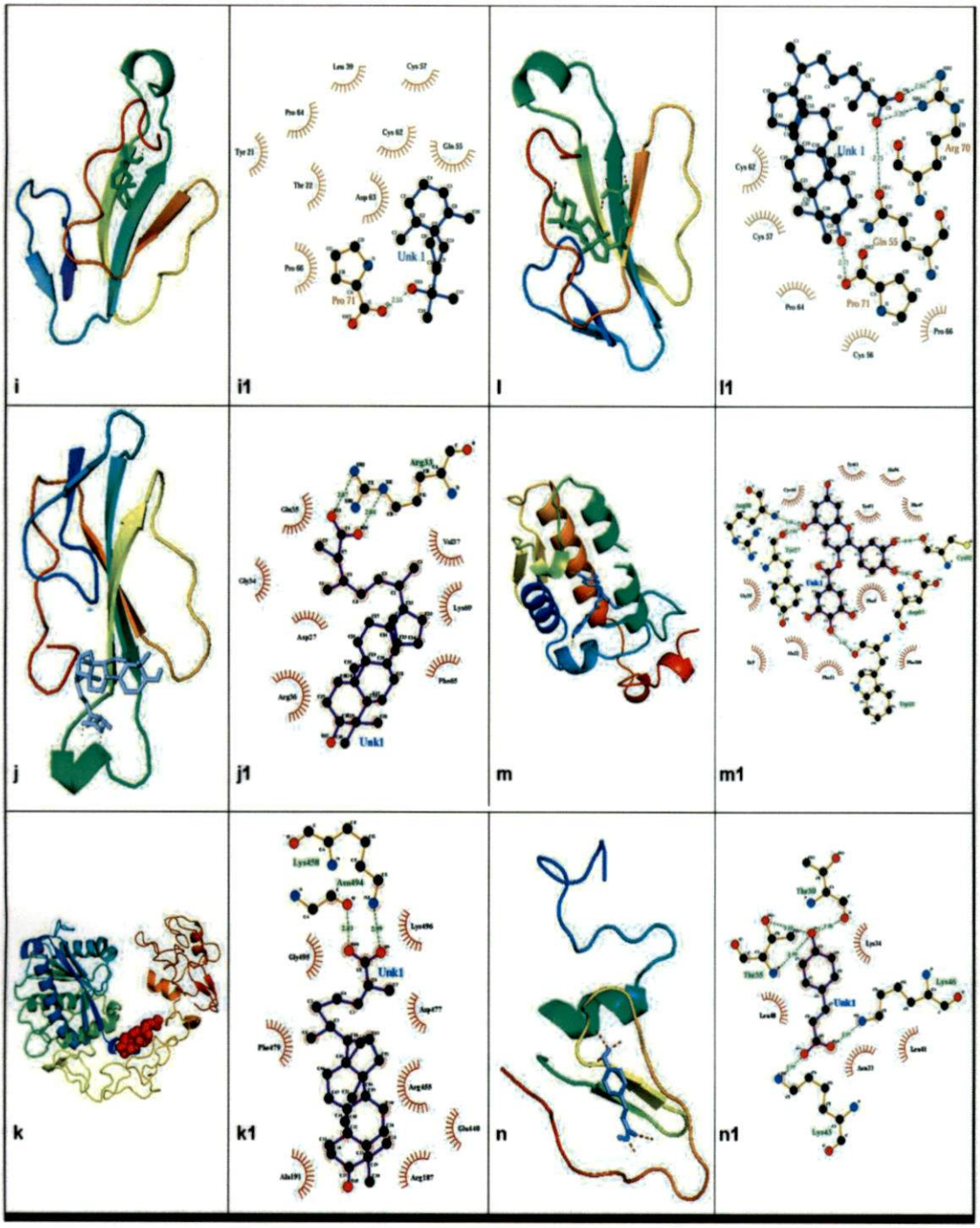


Figure 6 Continued. Docked structures of cobra venom proteins and lead molecule from *Mangifera indica* in PyMol and LigPlot respectively: (i and i1) LN3 and Hinesol (j and j1) LN5 and Ambolic acid (k and k1) PRT and Mangiferonic acid (l and l1) LN4 and Isomangiferolic acid (m and m1) PLA2 and Epicatechin-3-O-gallate (n and n1) SP and P-coumaric acid

4.4 DOCKED RESULT OF *TAMARINDUS INDICA* L.

Eighty phytochemicals from *Tamarindus indica* were docked with each of the 14 cobra venom proteins and the results revealed that the plant contains phytochemicals for inhibiting the activity of cobra venom proteins except long neurotoxin 2 and cobramine A. It was also noted that, of the 80 phytochemicals screened, 12 compounds have no inhibitory activity on the tested 14 venom proteins. Maximum number of inhibitory compounds was found against L-aminoacid oxidase (47) followed by phospholipase A2 (44) then comes cobrotoxin (11), proteolase (9), serine protease (8), long neurotoxin 1 (5), Long neurotoxin 3 (5), cobramine B (5), acetylcholineesterase (5), long neurotoxin 5 (4), cytotoxin 3 (2) and Long neurotoxin 4 (1) respectively. Details of the docked results are as follows.

Forty four hit molecules were obtained against phospholipase A2. Of these five molecules *viz.* benzyl benzoate (-8.17 kcal/mol), beta sitosterol (-11.45 kcal/mol), compesterol (-10.22 kcal/mol), cycloartanol (-9.72 kcal/mol) and isovetexin (-8.92 kcal/mol) were selected as lead molecules. The compound beta sitosterol was selected as the best lead since it has least binding energy and inhibition constant of 0.004 μM. The lead molecule also forms a hydrogen bond with Asn52 and hydrophobicaly interact with critical residue Asp48. Eleven hit molecules were noted against cobrotoxin. Of these, five compounds *viz.* compesterol (-6.09 kcal/mol), cycloartanol (-6.25 kcal/mol), lupanone (-7.05 kcal/mol), procyanidin dimer (-7.15 kcal/mol) and beta-amyrin (-6.26 kcal/mol) were selected as the lead. The compound procyanidin dimer with least binding energy and inhibition constant of 5.72 μM was selected as the best lead molecule. It forms seven hydrogen bonds with the residues Ser8, Thr37, Tyr35, Asp31 and critical residue Arg33.

Five compounds hit molecules were noted against long neurotoxin 1 and they were anthocyanin (-5.58 kcal/mol), benzyl benzoate (-5.04 kcal/mol), linalool (-5.22 kcal/mol), linonene (-5.49 kcal/mol) and safrole (-5.14 kcal/mol). The compound

benzyl benzoate was recommended as the best lead since it forms a hydrogen bond with Glu55 and hydrophobic interaction with the critical residue Thr22. Against Long neurotoxin 3, five lead molecules were obtained. They were anthocyanin (-5.72 kcal/mol), benzyl benzoate (-5.72 kcal/mol), linalool (-5.20 kcal/mol), linonene (-5.48 kcal/mol) and safrole (-5.15 kcal/mol). The compound linalool was selected as the best lead since it forms two hydrogen bonds with residues Pro71 and Glu55 and hydrophobic interaction with critical residue Tyr21. Only one inhibitory compound, luteolin was obtained against long neurotoxin 4. The binding energy value was -5.24 kcal/mol and inhibition constant was 145.4 μM. The compound also forms five hydrogen bonds with Pro66, Pro71, Arg70 and with critical residue Gln55. Long neurotoxin 5 was inhibited by four compounds such as anthocyanin (-5.37 kcal/mol), lupanone (-5.95 kcal/mol), taxifolin (-5.00 kcal/mol) and beta-sitosterol (-5.87 kcal/mol). The compound taxifolin was suggested as the best lead since it forms five hydrogen bonds with residues Thr67, Val37 and Lys69.

Five hit molecules were obtained against cobramine B. They were 1 malic acid (-5.80 kcal/mol), cinnamic acid (-5.14 kcal/mol), maleic acid (-5.76 kcal/mol), nicotinic acid (-5.19 kcal/mol) and tartaric acid (-6.38 kcal/mol). Among these, 1 malic acid was selected as the best lead since it forms six hydrogen bonds with residues Tyr22, Lys12, Lys18 and Cys38. Only two hit molecules such as anthocyanin (-5.30 kcal/mol) and beta sitosterol (-6.09 kcal/mol) were obtained against cytotoxin 3. The compound beta sitosterol was recommended as the best lead since it forms two hydrogen bonds with residues Arg36, Leu6 and hydrophobic interaction with the critical residue Lys18.

Nine hit molecules were obtained against proteolase and the selected lead molecules are compesterol (-6.01 kcal/mol), cycloartanol (-6.62 kcal/mol), lupanone (-7.25 kcal/mol), lupeol (-6.19 kcal/mol) and beta amyrin (-6.56 kcal/mol). The compound cycloartenol was suggested as the best lead since it forms hydrophobic interaction with critical residue Asp477. Five compounds hit molecules were obtained

against acetylcholine esterase. They were beta amyrin (-6.22 kcal/mol), compesterol (-6.14 kcal/mol), lupeol (-6.04 kcal/mol), cycloartanol (-5.41 kcal/mol) and lupanone (-5.14 kcal/mol). Among these the compound compesterol was selected as the best lead since it forms two hydrogen bonds with Gly78 and Glu75 and hydrophobic interaction with the critical residue Ser76. Against L aminoacid oxidase, five molecules *viz.* compesterol (-9.04 kcal/mol), cycloartanol (-9.06 kcal/mol), lupanone (-9.71 kcal/mol), lupeol (-9.70 kcal/mol) and beta-amyrin (-9.29 kcal/mol) were suggested as lead molecules. The compound compestrol was recommended as the best lead since it forms hydrogen bond with critical residue Arg90. Eight hit molecules were obtained against serine protease, among these, five compounds *viz.* cycloartenol (-5.86 kcal/mol), lupanone (-6.82 kcal/mol), lupeol (-5.80 kcal/mol), safrole (-5.38 kcal/mol) and beta amyrin (-6.74 kcal/mol) were suggested as lead molecules. The compound safrole was recommended as the best lead since it forms two hydrogen bonds with Thr39 and Thr35.

Table 5. Docked result of *Tamarindus indica*.

Target	Lead molecule	BE (kcal/ mol)	KI (μM)	H B	Bond type	Bond length
PLA2	Benzyl benzoate	-8.17	1.02	1	Gly29 NHO	2.82
	Beta sitosterol	-11.4	0.004	1	Asn52 NHO	3.04
	Compesterol	-10.2	0.032	0		
	Cycloartanol	-9.72	0.075	0		
	Isovetexin	-8.92	0.291	7	Trp18 OHO Gly29 NHO Asn52 NHO Gly32 OHO Asn49 OHO Gln45 OHO Cys44 OHO Asp48	2.56, 2.57, 2.91, 3.32, 3.20, 2.61, 2.73
CBT	Compesterol	-6.09	34.46	0		
	Cycloartanol	-6.25	26.26	2	Arg28 NHOThr37 OHO	2.64, 2.69
	Lupanone	-7.05	6.78	0		
	Procyanidin dimer	-7.15	5.72	7	Ser8 NHO Thr37 NHO, NHO Tyr35 OHO, OHO Asp31 OHO Arg33 OHO	3.05, 2.87, 3.20, 3.07, 3.01, 2.74, 2.87
	Beta amyrin	-6.26	25.92	1	Arg28 NHO	3.16
LN 1	Anthocyanin	-5.58	81.56	0		
	Benzyl benzoate	-5.04	200.8	1	Glu55 NHO	2.83
	Linalool	-5.22	148.6			
	Linonene	-5.49	94.52	0		
	Safrole	-5.14	170.2	0		
LN 2	-	-	-	-	-	-
LN 3	Anthocyanin	-5.72	63.97	0		
	Benzyl benzoate	-5.15	167.6	1	Glu55 NHO	2.7
	Linalool	-5.20	154.9	2	Pro71 OHO Glu55 NHO	2.90, 3.03
	Linonene	-5.48	95.42	0		
	Safrole	-5.15	167.2	0		
LN 4	Luteolin	-5.24	145.4	5	Pro66 OHO Pro71 OHO, OHO Arg70 OHO Gln55 OHO	3.5, 2.8, 2.6, 2.7, 3.4
LN 5	Anthocyanin	-5.37	116.4	0		
	Lupanone	-5.95	43.26	0		
	Taxifolin	-5.00	216.9	5	Thr67 OHO, OHO Val37 NHO , OHO Lys69 NHO	2.83, 3.05, 2.83, 2.89, 3.01
	Beta sitosterol	-5.87	50.16	0		
CA	-	-	-	-	-	-
CB	1 malic acid	-5.80	56.31	6	Tyr22 OHO Lys12 NHO Lys18 NHO Cys38 OHO, OHO, NHO	2.97, 3.25, 2.65, 3.14, 2.99, 2.60
	Cinnamic acid	-5.14	170.4	3	Lys12 NHOCys38 OHO Lys18 NHO	2.78, 2.58, 2.95
	Maleicacid	-5.76	59.66	4	Lys18 NHO Lys12 NHOCys38 NHO Lys35 NHO	3.14, 3.10, 3.19, 3.02
	Nicotinic acid	-5.19	156.4	3	Cys38 NHO Lys12 NHO Lys18 NHO	3.16, 2.54, 3.13

Table 5. Continued

	Tartaric acid	-6.38	21.03	0		
CYT3	Anthocyanin	-5.30	131.2	0		
	Beta sitosterol	-6.09	34.56	2	Arg36 OHO Leu6 NHO	2.5, 3.32
PRT	Compesterol	-6.01	39.12	0		
	Cycloartanol	-6.62	14.04	0		
	Lupanone	-7.25	4.84	0		
	Lupeol	-6.19	28.86	0		
	Beta amyrin	-6.56	15.54	0		
ACE	Compesterol	-6.14	31.82	2	Gly78 NHOGlu75 OHO	2.59, 2.79
	Cycloartanol	-5.41	108.7	1	Leu37 OHO	2.86
	Lupanone	-5.14	171.9	0		
	Lupeol	-6.04	37.08	1	Thr69 OHO	2.67
	Beta amyrin	-6.22	27.49	1	Phe69 OHO	2.69
LAAO	Compesterol	-9.04	0.235	1	Arg90 NHO	2.79
	Cycloartanol	-9.06	0.227	1	Arg109 NHO	3.05
	Lupanone	-9.71	0.076	2	Arg109 NHO, NHO	2.93, 3.3
	Lupeol	-9.70	0.078	0		
	Beta amyrin	-9.29	0.155	2	Gly106 OHO Lys343 NHO	2.78, 2.67
SP	Cycloartanol	-5.86	50.78	1	Lys34 NHO	2.79
	Lupanone	-6.82	10.09	1	Lys34 NHO	2.86
	Lupeol	-5.80	55.62	1	Lys34 NHO	2.74
	Safrole	-5.38	113.4	2	Thr39 OHO Thr35 NHO	2.68, 2.77
	Beta amyrin	-6.74	11.45	1	Thr29 OHO	2.48

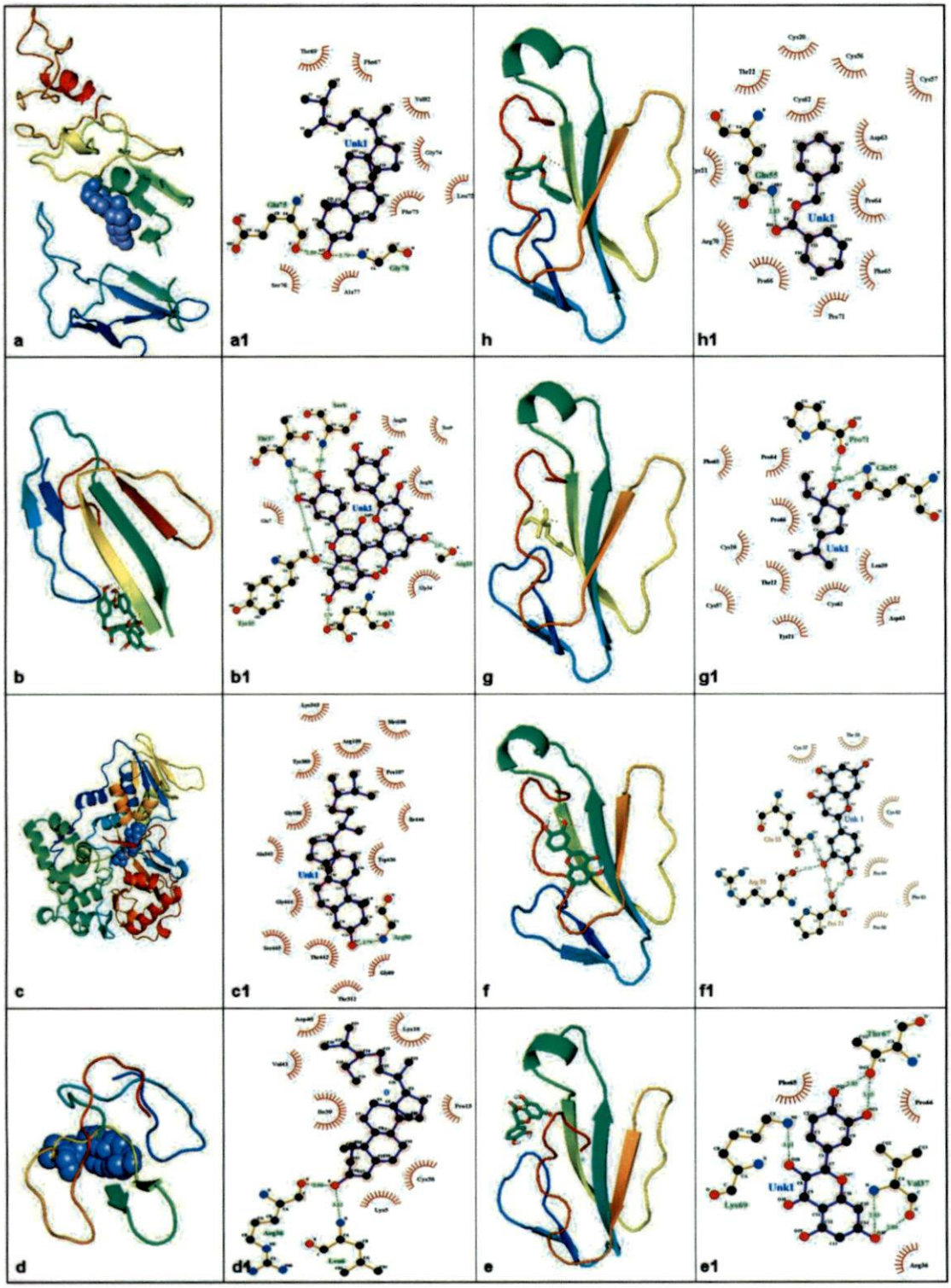


Figure 7. Docked structures of cobra venom proteins and lead molecule from *Tamarindus indica* in PyMol and LigPlot respectively: (a and a1) ACE and Compestrol (b and b1) CBT and Procyanidin dimer (c and c1) LAAO and Compestrol (d and d1) CYT3 and Beta sitosterol (e and e1) LN5 and Taxifolin (f and f1) LN4 and Luteolin (g and g1) LN3 and Linalool (h and h1) LN1 and Benzyl benzoate

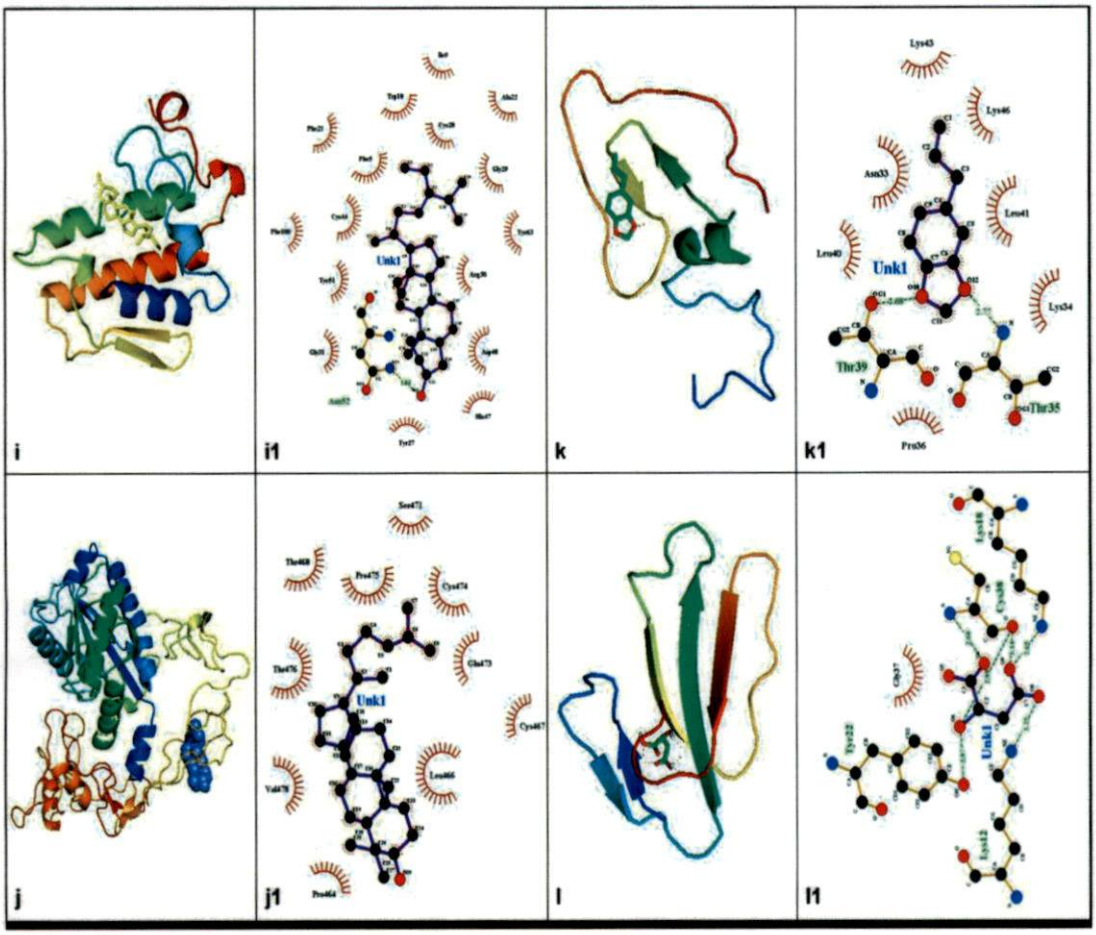


Figure 7 Continued. Docked structures of cobra venom proteins and lead molecule from *Tamarindus indica* in PyMol and LigPlot respectively: (i and i1) PLA2 and Beta-sitosterol (j and j1) PRT and Cycloartenol (k and k1) SP and Safrole (l and l1) CB and 1- malic acid

4.5 DOCKED RESULT OF *PHYLLANTHUS EMBLICA* L.

A total of 94 phytochemicals present in *Phyllanthus emblica* were docked with each of the 14 cobra venom target proteins and the results revealed that out of 94 compounds 24 phytochemicals do not show inhibitory activity. Maximum number of hit molecules obtained against phospholipase A2 (54) followed by L-aminoacid oxidase (35), proteolase (16), cobrotoxin (10), acetylcholineesterase (10), long neurotoxin 4 (9), long neurotoxin 5 (9), serine protease (8), cytotoxin 3 (5), cobramine B (3), long neurotoxin 2 (2), long neurotoxin 1 (1) and Long neurotoxin 3 (1). Hit molecule was not obtained against cobramine A. The selected lead molecules against each target protein were as follows.

Fifty four hit molecules ($\Delta G_{bind} \leq$-5.00 kcal/mol) were active against phospholipase A2. Five compounds having top least binding energy such as beta sitosterol (-10.70 kcal/mol), betulin (-10.67 kcal/mol), ursolic acid (-10.00 kcal/mol), betulinic acid (-9.97 kcal/mol) and betulonic acid (-9.97 kcal/mol) were selected as the lead molecules. Beta sitosterol was selected as the best lead molecule since it has a hydrogen bond with Asn52. Ten phytochemicals have inhibitory activity on cobrotoxin and five molecules showing top least binding energy such as friedelan-3-one (6.96 kcal/mol), beta amyrin ketone (-6.72 kcal/mol), lupeol acetate (-6.60 kcal/mol), betulonic acid (-6.42 kcal/mol) and Phyllanemblinins A (-6.07 kcal/mol) were selected as lead molecules. Among these, phyllanemblinins A was selected as the best lead since it has hydrogen bond with the critical residue Arg33.

Out of 94 phytochemicals only few of them showed inhibitory activity on the group of long neurotoxins. Only one compound, phyllantine (-5.61 kcal/mol) showed inhibitory activity on long neurotoxin 1 and this compound has no hydrogen bond interaction with the target molecule. Two phytochemicals *viz.* betulonic acid (-5.16 kcal/mol) and phyllantidine (-5.13 kcal/mol) showed inhibitory activity on long neurotoxin 2. Phyllantidine was selected as the best lead since it has a hydrogen bond

with the critical residue Lys23. Only one phytochemical, phyllantidine (-5.74 kcal/mol) showed inhibitory activity on long neurotoxin 3. While nine phytochemicals showed inhibitory activity on long neurotoxin 4 and five molecules having top least binding energy such as beta amyrin ketone (-6.19 kcal/mol), dihydrokaempferol (-5.35 kcal/mol), luteolin-4'oneohesperiodoside (-5.20 kcal/mol), epigallocatechin (-5.06 kcal/mol) and myo-inositol (-5.04 kcal/mol) were selected as lead molecules. Among these, epigallocatechin was selected as the best lead molecule since it has three hydrogen bonds with Thr22, Pro64, Gln 55 and one of the hydrogen bond formed with the critical residue Gln33. Nine phytochemicals have inhibitory effect on long neurotoxin 5 and five compounds *viz.* betulonic acid (-6.97 kcal/mol), ursolic acid (-6.94 kcal/mol), lupeol acetate (-6.31 kcal/mol), eriodictyol 7-o-glucoside (-5.86 kcal/mol) and chebulic acid (-5.34 kcal/mol) were selected as lead molecules. Of these, eriodictyol 7-o-glucoside was selected as the best lead since it has eight 8 hydrogen bonds with the residues Val37, Arg36, Arg33, Arg68 and Lys69 of protein. Of these, Arg33 is the critical residue.

Three pytochemicals *viz.* phyllantidine (-5.26 kcal/mol), beta humulene (-5.18 kcal/mol) and cinnamic acid (-5.14 kcal/mol) showed moderate inhibitory activity on cobramine B. Of these, cinnamic acid was selected as the best lead since it has three hydrogen bonds with Lys12, Cys38 and Lys18. Five compounds showed inhibitory activity on cytotoxin 3. They were betulonic acid (-6.73 kcal/mol), betulinic acid (-5.21 kcal/mol), coumaric acid (-5.21 kcal/mol), phyllantidine (-5.17 kcal/mol) and 3-ethylgallic acid (-5.07 kcal/mol). Of these, 3-ethylgallic acid was selected as the lead molecule since it forms four hydrogen bond with Arg36, Leu6 and Lys35.

Sixteen hit molecule were obtained against proteolase. Of these, five molecules having top least binding energy such as betulonic acid (-7.24 kcal/mol), lupeol acetate (-6.84 kcal/mol), phyllanemblinins B (-6.61 kcal/mol), ursolic acid (-6.40 kcal/mol) and friedelan-3-one (-6.37 kcal/mol) were selected as lead molecules. Betulonic acid was selected as the best lead molecule since it showed one hydrogen bond with Lys458

and hydrophobic interaction with the critical residue Asp477. Ten phytochemicals showed inhibitory activity on acetylcholine esterase and five molecules such as friedelan-3-one (-6.28 kcal/mol), lupenone (-6.25 kcal/mol), lupeol (-6.04 kcal/mol), phyllantine (-5.69 kcal/mol) and beta amyrin ketone (-6.15 kcal/mol) were selected as lead molecules. Of these, lupeol was selected as the best lead since it has the least binding energy, inhibition constant and form a hydrogen bond with Thr69. Thirty five phytochemicals showed inhibitory activity on L-aminoacid oxidase. Of these, five compounds having top least binding energy *viz.* beta amyrin ketone (-11.21 kcal/mol), betulin (-10.27 kcal/mol), eriodictyol 7-o-glucoside (-9.21 kcal/mol), ursolic acid (-9.91 kcal/mol), and beta amyrin-3-palmitate (-9.21 kcal/mol) were selected as lead molecules. Betulin was selected as the best lead molecule since it has five hydrogen bonds with Arg109, Met108, Ser445 and Gly444. Betulin also have hydrophobic interaction with the critical residue Arg95. Eight hit molecules were noted against serine protease. Among these, five molecules such as beta amyrin ketone (-6.79 kcal/mol), betulonic acid (-6.10 kcal/mol), eriodictyol 7-o-glucoside (-5.27 kcal/mol), lupeol acetate (-5.26 kcal/mol) and friedelan-3-one (-5.17 kcal/mol) were selected as the lead molecules. The compound eriodictyol 7-o-glucoside was selected as the best lead since it has seven hydrogen bonds with Lys46, Leu51, Lys28, Pro49 and Thr29 residues of the target protein molecule.

Table 6. Docked result of *Phyllanthus emblica*.

Target	Lead molecule	BE (kcal/ mol)	KI (μM)	H B	Bond type	Bond length
PLA2	Beta- sitosterol	-10.7	0.014	1	Asn52 NHO	3.11
	Betulin	-10.6	0.015	1	Asn52 OHO	3.11
	Betulinic acid	-9.97	0.049	2	Tyr63 OHOAsn52 OHO	2.77, 2.89
	Betulonic acid	-9.98	0.048	1	Tyr27 OHO	2.87
	Ursolic acid	-10.0	0.046	1	Tyr27 OHO	3.13
CBT	Betulonic acid	-6.42	19.84	0		
	Friedelan-3-one	-6.96	7.94	0		
	Lupeol acetate	-6.60	14.65	0		
	Phyllanemblinins A	-6.07	35.60	8	Asp31 OHO, OHOArg33 OHO, OHOArg28 NHO, NHO Thr37 OHO, Ser8 OHO	2.77, 2.47, 2.69, 3.12, 2.72, 2.86, 2.86, 2.54
	B-Amyrin ketone	-6.72	11.92	0		
LN 1	Phyllantine	-5.61	76.84	0		
LN 2	Betulonic acid	-5.16	164.6	1	Arg49 NHO	3.25
	Phyllantidine	-5.13	175.	1	Lys23 NHO	3.05
LN 3	Phyllantine	-5.74	61.56	0		
LN 4	Dihydro kaempferol	-5.35	120.1	3	Pro71 OHOThr22 OHOPro64 OHO	2.64, 2.76, 2.82
	Epigallocatechin	-5.06	194.8	3	Thr22 OHOPro64 OHO Gln55 OHO	2.53, 2.75, 2.67
	Luteolin-4'Oneohesperiodoside	-5.20	154.3	0		
	Myo-inositol	-5.04	201	0		
	B-Amyrin ketone	-6.19	28.99	1	Thr59 OHO	2.76
LN 5	Betulonic acid	-6.97	7.82	2	Lys69 NHO Arg36 NHO	3.06, 3.06
	Chebulic acid	-5.34	121.62	3	Asp27 OHO Arg33 NHO Val37 NHO	2.67, 2.72, 3.09
	Eriodictyol 7-O-glucoside	-5.86	50.24	8	Val37 OHO, OHO Arg36 OHO Arg33 OHO Arg68 NHO, NHO Lys69 NHO, NHO	2.68, 2.85, 3.18, 2.67, 3.00, 3.20, 2.66, 2.79
	Lupeol acetate	-6.31	23.54	2	Arg36 NHO, NHO	2.97, 3.20
	Ursolic acid	-6.94	8.13	2	Val37 NHO Thr67 OHO	3.09, 3.07
CA	-	-	-	-	-	-
CB	Cinnamicacid	-5.14	170.4	3	Lys12 NHO Cys38 OHOLys18 NHO	2.78, 2.58, 2.95
	Phyllantidine	-5.26	139.3	0		
	Beta Humulene	-5.18	159.1	0		
CYT3	3-ethylgallic acid	-5.07	193.46	4	Arg36 OHO, OHOLeu6 NHO, Lys35 NHO	2.66, 2.80, 2.88, 2.80
	Betulinic acid	-5.21	151.6			

Table 6. Continued

	Betulonic acid	-6.73	11.74	0		
	Coaric acid	-5.21	152.7	1	Lys35 OHO	2.67
	Phyllantidine	-5.17	162.7	2	Leu1 NHO, NHO	3.08, 2.98
PRT	Betulonic acid	-7.24	4.90	1	Lys458 NHO Asp477	2.63
	Friedelan-3-one	-6.37	21.40	0		
	Lupeol acetate	-6.84	9.62	0		
	Phyllanemblinins B	-6.61	14.22	12	Lys458 NHO Asp477 OHO, OHO, NHO, NHO Arg455 NHO Pro475 OHOCys474 NHO Glu440 NHO Lys387 NHO Leu385 OHO, OHO	2.90, 2.82, 2.97, 3.00, 2.94, 3.23, 2.63, 3.24, 3.12, 3.34, 2.87, 3.19
	Ursolic acid	-6.40	20.19	3	Lys458 NHO Glu439 OHO Cys474 NHO	3.14, 3.07, 3.10
ACE	Friedelan-3-one	-6.28	24.95	0		
	Lupenone	-6.25	26.29	0		
	Lupeol	-6.04	37.08	1	Thr69 OHO	2.67
	Phyllantine	-5.69	67.25	1	Ala77 NHO	2.96
	B-Amyrin ketone	-6.15	31.07	0		
LAAO	Betulin	-10.2	0.029	5	Arg109 NHO, OHO Met108 NHO Ser445 OHO Gly444 OHO	3.11, 3.07, 3.09, 3.07, 3.23
	Eriodictyol 7-O-glucoside	-9.21	0.177	7	Gly106 OHO Lys343 NHO Arg109 OHO, NHO Met108 NHO Tyr389 OHO	3.10, 3.12, 3.26, 2.81, 2.66, 2.90, 2.92
	Ursolic acid	-9.91	0.054	1	Arg109 NHO	2.81
	Beta Amyrin ketone	-11.2	0.006	1	Arg109 NHO	2.78
	Beta Amyrin-3-palmitate	-9.21	0.177	0		
SP	Betulonic acid	-6.10	33.71	1	Lys34 NHO	3.21
	Eriodictyol 7-O-glucoside	-5.27	136.2	7	Lys46 NHO Leu51 OHO Lys28 OHO, OHO Pro49 OHO, OHO Thr29 OHO	2.6, 2.9, 2.74, 2.9, 3.03, 2.96, 3.32
	Friedelan-3-one	-5.17	163.5	0		
	Lupeol acetate	-5.26	140.0	1	Lys28 NHO	2.89
	Beta Amyrin ketone	-6.79	10.47	0		

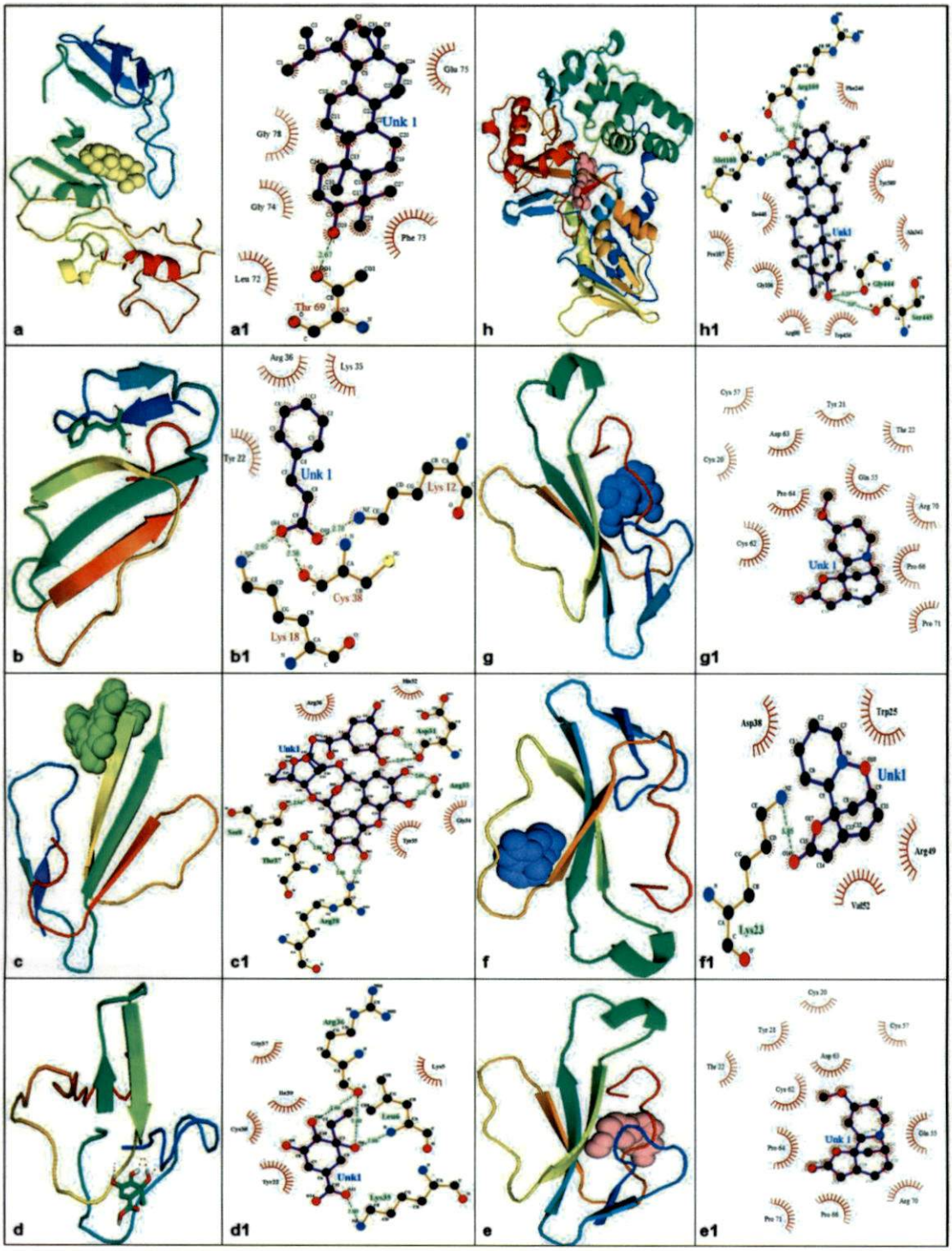


Figure 8. Docked structures of cobra venom proteins and lead molecule from *Phyllanthus emblica* in PyMol and LigPlot respectively: (a and a1) ACE and Lupeol (b and b1) CB and Cinnamic acid (c and c1) CBT and Phyllemblinins A (d and d1) CYT3 and 3-Ethyl ellagic acid (e and e1) LN3 and Phyllantine (f and f1) LN2 and Phyllantidine (g and g1) LN1 and Phyllantine (h and h1) LAAO and Betulin

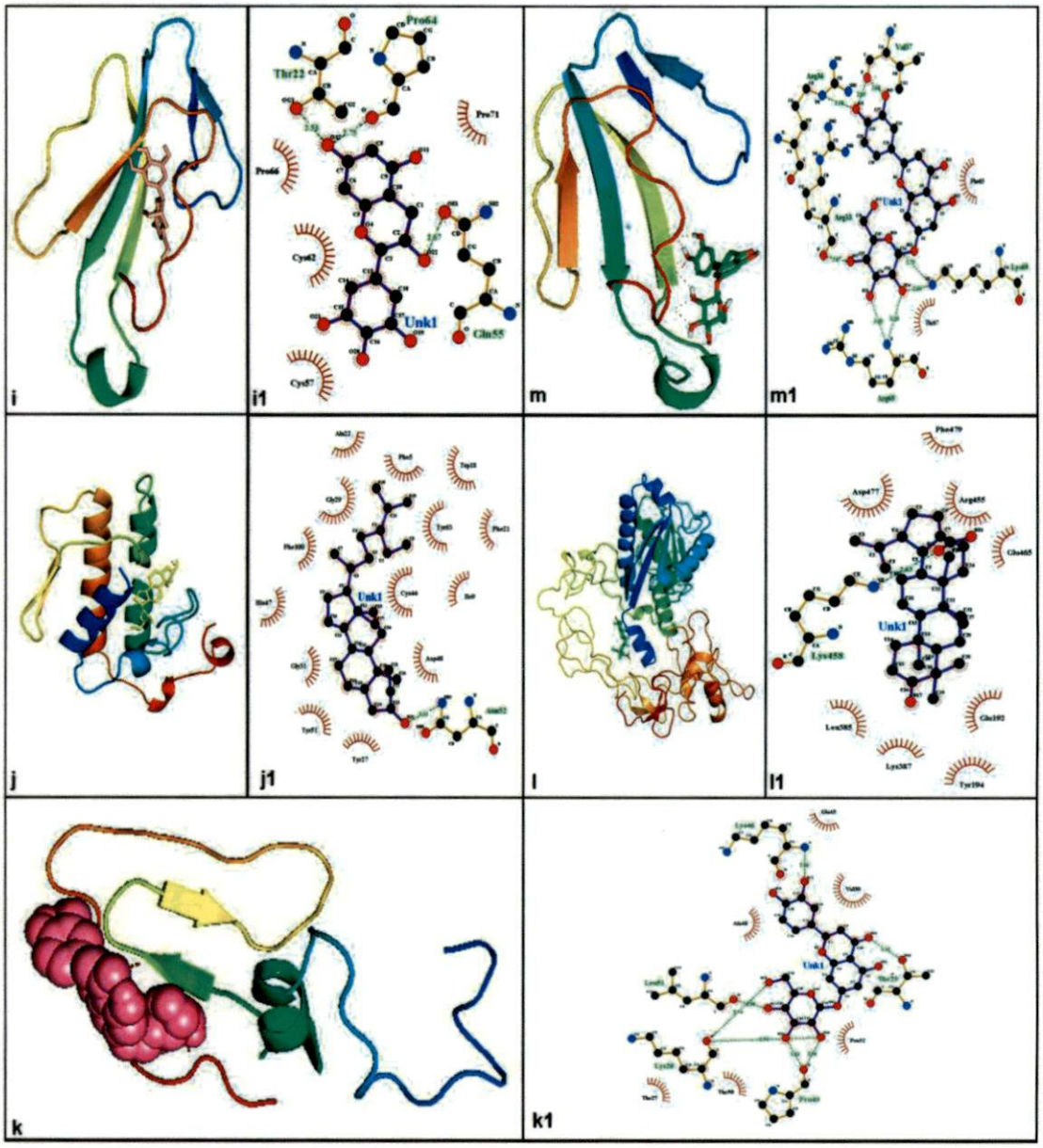


Figure 8 Continued. Docked structures of cobra venom proteins and lead molecule from *Phyllanthus emblica* in PyMol and LigPlot respectively: (i, and i1) LN4 and Epigalocatechin (j and j1) PLA2 and Beta-sitosterol (k and k1) SP and Eriodictyol-7-O-glucoside (l and l1) PRT and Betulonic acid (m and m1) LN5 and Eriodictyol-7-O-glucoside

IDENTIFICATION OF LEAD MOLECULE TROUGH DST METHOD

To find out the best lead molecules against each venom protein from 522 phytomolecules derived from the five selected plants and to avoid errors in such selection, the five top ranked lead molecule obtained in AutoDock were again docked with each of the 14 cobra venom protein such as phospholipase A2, cobrotoxin, long neurotoxin 1, long neurotoxin 2, long neurotoxin 3, long neurotoxin 4, long neurotoxin 5, cobramine A, cobramine B, Cytotoxin 3, proteolase, acetylcholine esterase, l aminoacid oxidase and serine protease using the docking tools, Hex Server, PatchDock and iGEMDOCK. The results obtained in all the four docking tools were analyzed through DST method and determined the best lead molecules, which are shown in table.

Table 7. Docking result using various softwares and lead identified through DST method.

Venom protein	Lead molecule	AutoDock (kcal/mol)	PatchDock (kcal/mol)	Hex Server (kcal/mol)	iGEMDOCK (kcal/mol)
PLA2	Gamma sitosterol	-11.52	4948	-290.22	-89.08
CBT	Procyanidin dimer	-7.15	3922	-274.36	-135.88
LN 1	7-epi-a-selinene	-6.06	2858	-170.63	-54.42
LN 2	Betulonic acid	-5.16	4054	-243.46	-70.03
LN 3	7-epi-a-selinene	-6.1	2928	-170.45	-54.42
LN 4	Mangiferolic acid	-5.87	4502	-256.95	-71.48
LN 5	Ambolic acid	-6.45	4194	-263.92	-72.01
CA	1-epi-cubenol	-5.7	2856	-170.08	-51.72
CB	Phyllantidine	-5.26	3080	-208.94	-60.96
CYT 3	Isomangiferolic acid	-7.66	4116	-264	-98.33
PRT	Mangiferonic acid	-6.57	5968	-309.69	-89.18
ACE	Beta sitosterol	-6.93	4602	-260.81	-74.18
LAAO	Mangiferolic acid	-11.08	6474	-322.24	-91.23
SP	Mangiferonic acid	-6.86	5398	-303.54	-85.35

DISCUSSION

5. DISCUSSION

Snake envenomation is a global serious health concern especially in tropical and sub-tropical regions (Nisha *et al.*, 2014). It was estimated that the average global incidence of snakebites is 5.0 million per year, of these, 3.75 million people were severely affected and 1.25 million died. India ranks top first in snakebite death rate with annually 20000-50000 people (Chippaux, 1998; Kasturiratne *et al.*, 2008, Williams *et al.*, 2010). These estimates are arbitrary as majority of death are unreported (Gupta and Peshin, 2014). Immunotherapy is the only treatment against snake envenomation in modern medicine but it has several limitations such as serious side effects like serum sickness reactions, anaphylactics shock, pyrogen reaction, etc. Non-availability of antivenom, identification of snake species and use of monovalent instead of polyvalent antivenom are other problems and all these are well described by Nisha *et al.* (2014). Snake venom is a complex mixture of biomolecules such as proteins, lipids, nucleotides, inorganic ions etc., of these, 90% constitutes proteins. Majority of the protein molecules are harmless and used as drugs but few of them are toxic. About 20 toxic proteins were reported from different snake species, of these, 6-12 are common. In India majority of the death is caused by four species *viz.* Indian cobra (*Naja naja*), *Russell's viper (Daboia russelii*) krait (*Bungarus caeruleus)* and saw-scaled viper (*Echis carinatus).* Among these, Indian cobra cause high rate of mortality and the death is mainly caused due to the dysfunction of central nervous system (Binh *et al.*, 2010). In fact, krait venom has ten times more neurotoxic potential than Indian cobra venom but the lethality rate caused by Indian cobra is comparatively high. This may be due to the presence of long curved sharp fangs in cobra which can inject the venom into the vascular tissue and also the venom contains hyaluronidase which enhances spreading the venom rapidly and cause death within short time though cobra venom is less toxic than viper and krait (Sreekumar *et al.*, 2014). Fourteen proteins of cobra venom, which induces neurotoxicity, myotoxicity, hemolysis, coagulopathy, renal failure, severe necrosis, hypopituitarism, etc. were selected as target proteins.

Modern medicine is targeted on a single cause protein and therefore, formulation of a single drug against multifactorial causation like a complex mixture of venom proteins is a difficult task (Nisha *et al.*, 2016).

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 (Clark 2008, Ripphausen *et al.*, 2011). Many authors have demonstrated that *in silico* screening followed by *in vitro* and *in vivo* screening are the best methods to find out potential lead molecules (Sakthivel *et al.*, 2013). Leanpolchareanchai (2009) conducted *in vitro*, *in silico* and *in vivo* experiments and demonstrated the anti-snake venom activity of Thai mango (*Mangifera indica* L.). Among the *in silico* screening methods docking has been widely used for the identification of lead molecules and its significances are well discussed (Kitchen *et al.*, 2004; Cheng *et al.*, 2012). Many authors had utilized the technique for the identification of lead molecules from Indian medicinal plants, which have been used against disease like tuberculosis (Haridas *et al.*, 2016) hepatitis B (Mathew *et al.*, 2016, Shefin *et al.*, 2016) and snakebites in Indian systems of medicine (Nisha *et al.*, 2014; Sreekumar *et al.*, 2014). Therefore, in the present investigation docking method was used for determining the efficacy of anti-snake venom plants and identification of lead molecules. Success of the drug discovery process depends on the identification of the right target protein for *in silico* screening, the 3D structures of the target proteins are to be procured from available databases like PDB or to be created through a series of process such as sequence analysis, determination of closely related sequence, 3D structure modeling and its validation. In the case of Indian cobra venom all these processes were well done and screened several plant derived molecules by Sreekumar *et al.* (2014), and Nisha *et al.* (2016). Therefore, the same protein 3D structures were used for the present investigation.

Preparation of the ligand molecules is the next step in docking process and in the present study phytochemicals derived from five fruit plants such as *Punica granatum*, *Syzygium cumini*, *Phyllanthus emblica*, *Mangifera indica* and *Tamarindus indica* were selected for docking. All the selected five plants have been used for the treatment of many diseases including snakebite (Dey and De, 2012). These plants are commonly used as food, vegetables, spices and medicine. Exploration of medicinal properties in nutraceuticals attained special attention since its administration may prevent or cure diseases and in such a way the use of medicine in pure form can be limited or avoided. As followed in earlier reports (Dey and De, 2012) the structure of phytochemicals derived from the selected plants were procured from various sources such as literature and open access chemical databases such as Pubchem, ChemSpider. Out of total 522 phytochemicals derived from five selected plants, except three molecules, all others were retrieved from chemical databases like PubChem since they are freely available. The structure of the remaining three molecules structures were drawn using freely available tool, ChemSketch which is an open access user friendly tool. The 2D structures of phytochemicals were converted into 3D structure using the tool CORINA, which is an open access widely used tool for 3D structure generation of small and medium sized chemical molecules especially drug molecules. Several docking tools based on different algorithms are available for docking. However, among the published papers in this line 70% authors used AutoDock tool (Mihasan, 2012). It is an open access first docking tool to model the ligand with full conformational facility and its running procedure, advantages and limitations are well explained by Morris *et al.* (2009). Structural investigation, detection of active site, right binding residues and pharmacophore nature of the target molecules are the key factors for the successful docking which leads to identification of the best lead molecules. It can be achieved by close observation of the target molecules using protein visualization and active site detection tools. For molecular visualization, the tools PyMol and LigPlot were used. All these tools are open access and widely used and analysis of the molecules using more than one tools generate more accuracy.

Generally, natural compounds are exception to Lipinski rule of five (Ganesan, 2008), however, due to the limitation in AutoDock tool, compounds with molecular weight more than 1000 Da were excluded.

Generally, lead molecules are selected based on three criteria, they are (1) Free energy of binding, which is a measure of the affinity of ligand-protein complex, or is the difference between the energy of complex and the sum of energies of each molecule separately. A docked molecule with the least free energy of binding was always considered as the best lead. (2) Inhibition constant, which is an indication of how potent an inhibitor is, it is the concentration required to produce half maximum inhibition. (3) H bond, which is the strongest type of intermolecular force or dipole interaction make the binding force more stable. As a general principle the docked structures having ΔG_{bind} less than -5 kcal/ mol were selected as hits or promising lead molecules. In order to find out the molecular interaction of the docked structure the tool LigPlot was used, in addition to H-bond interaction this tool clearly indicated other interactions such as hydrophobic, hydrophilic, van der Waals dispersion forces etc.

The analysis of the docked results between 147 phytochemicals derived from *Punica granatum* and each of the selected 14 cobra venom proteins in AutoDock revealed that the plant can inhibit all cobra venom proteins except CA. The selected best lead molecules against each protein were beta sitosterol against acetylcholinesterase and CYT 3, tartaric acid against CB, lupenone against cobrotoxin, ursolic acid against L amino acid oxidase, para- menth-1 en-4-ol against LN 1. The compound sedridine also showed similar binding energy but its inhibition constant was high (94.24 μM) when compared to the former (87.36 μM) and has hydrophobic interaction with the critical residue Thr22. 4n- propylresorcinol against LN 2 and LN 3, estriol against LN 4, ethyl-brevifolin carboxylate against LN 5, gamma sitosterol against PLA 2, cycloartenol acetate against PRT oxandrolone against SP. The result indicated that the plant have anti-cobra venom activity. A total 95 compounds from *Syzygium cumini* were screened against each of the cobra venom protein and found that all of them were inhibitory to the cobra venom proteins except LN 2. The selected best

lead molecules against each protein was betulinic acid against ACE and LAAO. Only one compound showed activity against CA and it was calacorene. Citic acid against CB, epifriedelanol against cobrotoxin, alpha cadinol against CYT 3, alpha santalol against LN 1 and LN 5, alpha terpineol against LN 3, cyanidin against LN 4, epi-friedlanol against PLA 2, and betulinic acid against SP. The docked results revealed that the plant has potential inhibitory activity on cobra venom.

A total of 100 phytochemicals from *Mangifera indica* were screened and the docked results in AutoDock revealed that the plant can inhibit the activity of all cobra venom proteins. The best lead molecules selected protein was mangiferolic acid against acetylcholinesterase, 1-epi-cubenol against CA, ambonic acid against cobrotoxin and LN 5, isomangiferolic acid against CYT 3, isomangiferolic acid against L amino acid oxidase, hinesol against LN 1 and LN 3, guaiol against LN 2, isomangiferolic acid against LN 4, epicatechin-3-O gallate against PLA 2, mangiferonic acid against PRT and para coumaric acid against SP. The binding energy level obtained from this plant was comparatively very low and this plant can be suggested as the best source of cobra venom inhibitors. A total of 80 phytochemicals from *Tamarindus indica* were screened and found that the plant can inhibit all cobra venom toxic proteins except CA and LN 2. The best lead molecules selected were compestrol against acetylcholinesterase, 1-malic acid against CB, procyanidin dimer against cobrotoxin, beta sitosterol against CYT 3, compestrol against L amino acid oxidase, benzyl benzoate against LN 1, linalool against LN luteolin against LN 4, taxifolin against LN 5, beta sitosterol against PLA 2, cycloartenol against PRT and safrole against SP. This plant also shows potential inhibitory activity on cobra venom.

A total of 94 phytochemicals from *Phyllanthus emblica* were screened and found that all of the target proteins except CA, were inhibited by the plant. The best lead molecules selected against each target protein were lupeol against acetylcholine esterase, cinnamic acid against CB, phyllemblinins against cobrotoxin, 3- ethylgallic acid against CYT 3, betulin against L aminoacid oxidase, phyllantine against LN 1 and

LN 3, phyllantidine against LN 2, epigallocatechinis against LN 4, eriodictyol 7-O-glucoside against LN 5, beta sitosterol against PLA 2, betulonic acid against PRT and eriodictyol 7-O- glucoside against SP.

The overall docking results in AutoDock indicated that among the five plants screened, *Mangifera indica* is able to inhibit all the 14 cobra venom proteins. The anti-snake venom activity of *Mangifera indica* was reported earlier by Leanpolchareanchai *et al.* (2009) but in their report based on the *in vitro* and *in vivo* study results they have demonstrated the molecular interaction of three phenolic compounds such as penta-o-galloyl-β-D-glucopyranose, methyl gallate and gallic acid with the venom proteins PLA 2 and LAAO of *Calloselasma rhodostoma* Kuhl (Malayan pit viper) and *Naja naja kaouthia* Lesson (Thai cobra). While in the present study, all the phytochemicals so far reported from *Mangifera indica* having molecular weight up to 1000 Da were docked with all the 14 toxic Indian cobra venom proteins to find out the best lead molecules against each venom protein. It was also noted that in the earlier reports the active compounds *viz.* penta-o-galloyl-β-D-glucopyranose, methyl gallate and gallic acid showed only moderate binding energy with the target molecules PLA 2 and LAAO. In the present investigation, all the reported compounds were screened to find out more potent lead molecules having least binding energy. In this back drop, among the five plants *Mangifera indica* can be recommended as the best cobra anti-venom plant. Other plants also contain phytochemicals for inhibiting majority of the cobra venom proteins. The plant *Punica granatum, Phyllanthus emblica* and *Tamarindus indica* have no inhibitory compounds against CA and *Syzygium cumini* and *Tamarindus indica* have no inhibitory compound against LN 2. LNs are the group of finger protein family and share common structural features and therefore, the compounds inhibiting other neurotoxin may inhibit the activity of LN 2 also. Therefore, *Syzygium cumini* can also be recommended as anti-cobra venom plant. Among the three cardiotoxins or cytotoxins such as CA, CB and CYT 3, other three plants (*Punica granatum, Phyllanthus emblica* and *Tamarindus indica*) did not show any inhibitory activity

against CA. The structural features of CA and CB are almost similar. These proteins are heat stable but less toxic (Binh *et al.*, 2010*)*. CA and CB are similar with respect to their ability to inhibit iodide accumulation, behavior on carboxymethyl cellulose, heat stability, isoelectric point, and molecular weight. This suggests that a structural relation exists between these two proteins. Furthermore, cobrotoxin is readily hydrolyzed by trypsin. These proteins are also enhancing the lytic activity of PLA 2. Considering the less toxicity and structural similarity with CB, the natural compounds synergetic effect may neutralise the toxicity of CA also. In the light of these, all these three plants may also recommend as anti-cobra venom plants.

As reported by many authors (Sreekumar *et al.*, 2014) several compounds showed inhibitory activity on more than one venom proteins. In the case of *Punica granatum* beta sitosterol, cycloartenol acetate, esterone and gamma sitosterol showed inhibitory activity against eight venom proteins. Similarly, phytochemicals from *Syzygium cumini* such as cyanidin, epifriedelanol, friedelin and alpha copaene showed inhibitory activity on six target proteins. Phytochemicals from *Phyllanthus emblica* such as beta sitosterol and betulonic acid showed inhibitory activity on eight target proteins and another compound phyllantidine inhibited 10 target proteins. In the case of *Mangifera indica*, the compound named guaiol inhibited 11 target molecules. Other compounds like isomangiferolic acid, mangiferolic acid and mangiferonic acid inhibited 9 targets and ambolic acid and ambonic acid inhibited 8 target molecules respectively. In *Tamarindus indica,* beta sitosterol inhibited 8 target proteins. Anthocyanin inhibited 7 target proteins and compestrol, cycloartanol and lupanone inhibited six target molecules. Many natural compounds showed similar multi protein inhibitory activity and synergistic effect of all these compounds together act as a strong barrier against cobra venom toxicity. Different docking tools following different algorithms and scoring methods like AutoDock showed several lead molecules with negligible difference in binding energy were obtained. In this backdrop, to avoid errors during the selection of the best lead molecules from a total of 522 phytochemicals

derived from five selected plants against each of the 14 cobra venom proteins, top ranked five lead molecules having least binding energy were again docked using different tools based on different algorithms and scoring method such as PatchDock, Hex Server and iGEMDOCK.

The docked results obtained in four docking tools were statistically analysed following Dempster Shafer Theory (DST) and identified the best lead molecules from the screened five plants. The best lead molecules identified through DST analysis were gamma sitosterol (-11.52 kcal/mol) from *Punica granatum* against PLA 2, procyanidin dimer (-7.15 kcal/mol) from *Tamarindus indica* against cobrotoxin, 7-epi-a-selinene (-6.06 kcal/mol) from *Mangifera indica* against LN 1, betulonic acid (-5.16 kcal/mol) from *Phyllanthus emblica* against LN 2, 7-epi-a-selinene (-6.1 kcal/mol) from *Mangifera indica* against LN 3, mangiferolic acid (-5.87 kcal/mol) from *Mangifera indica* against LN 4, ambolic acid (-6.45 kcal/mol) from *Mangifera indica* against LN 5, 1-epi-cubenol (-5.7 kcal/mol) from *Mangifera indica* against CA, phyllantidine (-5.26 kcal/mol) from *Phyllanthus emblica* against CB, isomangiferolic acid (-7.66 kcal/mol) from *Mangifera indica* against CYT 3, mangiferonic acid (-6.57 kcal/mol) from *Mangifera indica* against PRT, beta sitosterol (-6.93 kcal/mol) from *Punica granatum* against ACE, mangiferolic acid (-11.08 kcal/mol) from *Mangifera indica* against LAAO, mangiferonic acid (-6.86 kcal/mol) against SP respectively. The DST analysis revealed that the best lead molecules against nine proteins such as LN 1, LN 3, LN 4, LN 5, CA, CYT 3, PRT, LAAO and SP were obtained from the plant *M. indicia,* the best lead against ACE and PLA2 were obtained from the plant *P. granatum*, the best lead against LN 1 and CB were obtained from the plant *P. emblica* and the best lead against cobrotoxin was obtained from *T. indica*.

In silico screening is the best option to identify the best lead molecule based on molecular interaction analysis. However, false positive and false negative results may be encountered when selection is made based on theoretical interpretation. Therefore, *in vivo* and *in vitro* experimental demonstrations are inevitable for further confirmation.

SUMMARY

6. SUMMARY

Snakebite which lead to consequent mortality and morbidity is a serious global health issue particularly in tropical countries like India. Immunotherapy is the only treatment against snake envenomation in modern medicine but it has inherent with several limitations. Herbal medicines are widely used for the treatment of snakebite and it is believed that it may induce less or no side effects to the victims. But its efficacy and mode of drug action are seldom investigated scientifically. It is well acknowledged that *in silico* screening of phytomolecules against venom protein is the best option to validate the efficacy and demonstrate molecular mechanism of drug action in a rapid and economic way. Among the venomous snake species in India *Naja naja* (Indian cobra) caused high rate of mortality and several plants including *Punica granatum* (L.), *Syzygium cumini* (L.), *Mangifera indica* (L.), *Tamarindus indica* (L.) and *Phyllanthus emblica* (L.) have been used against snakebites particularly for the detoxification activity of cobra venom. In the light of these in the present investigation 14 cobra venom toxic proteins such as phospholipase A2, cobrotoxin, long neurotoxin 1, long neurotoxin 2, long neurotoxin 3, long neurotoxin 4, long neurotoxin 5, cobramine A, cobramine B, cytotoxin 3, proteolase, acetylcholineesterase, L-aminoacid oxidase and serine protease were selected as the target molecules and a total of 522 phytochemicals from the above mentioned five plant species were selected as ligand molecules.

The 3D structures of the target proteins were procured from earlier authors reports. For molecular visualization of the target the tools PyMol was used. A total of 522 phytochemicals (*Punica granatum* - 147, *Syzygium cumini* - 95, *Mangifera indica* - 100, *Tamarindus indica* - 80 and *Phyllanthus emblica* - 94) were selected as ligand for docking. Of these, structures of 519 phytochemicals were retrieved from databases and for the remaining three molecules structures were created using the tool ChemSketch and its 3D structures were created in CORNIA.

All the selected phytochemicals were docked into the binding site of these 14 target protein using the docking tool, AutoDock 4.2. The docked structure having binding energy value ≤ -5 kcal/mol were selected as the hit molecule and from these hit molecule, best lead molecule were selected based on the hydrogen bond formed, least binding energy and hydrophobic interaction with the critical residue.

The results showed that *Mangifera indica* can inhibit all the 14 cobra venom proteins, *Punica granatum, Phyllanthus emblica* and *Tamarindus indica* have no inhibitory compounds against cobramine A and *Syzygium cumini* and *Tamarindus indica* have no inhibitory compound against long neurotoxin 2.

In order to find out best lead molecule without error against each venom protein from 522 phytomolecules, top ranked five lead molecules were again docked using the tools Hex Server, PatchDock and iGEMDOCK and the results were subjected to DST analysis and selected the best lead molecules from *Mangifera indicia* against 9 proteins. *Punica granatum* and *Phyllanthus emblica* has lead molecule against 2 proteins and *Tamarindus indica* have lead molecule against 1 protein. The best lead from *Mangifera indicia* were 7-epi-a-selinene (-6.06 kcal/mol) on long neurotoxin 1 and long neurotoxin 3, mangiferolic acid on long neurotoxin 4, ambolic acid on long neurotoxin 5, 1-epi-cubenol on cobramine A, isomangiferolic acid on cytotoxin 3, mangiferonic acid on proteolase and serine protease and mangiferolic acid on L-amino acid oxidase. The two best lead obtained from *Punica granatum* were gamma sitosterol on phospholipase A2 and beta sitosterol on acetylcholineesterase. Similarly from *Phyllanthus emblica* were betulonic acid on long neurotoxin 2 and phyllantidine on cobramine B and the only one best lead obtained from *Tamarindus indica* was procyanidin dimer on cobrotoxin. It was also noted that many lead molecules have multi-protein inhibitory activity.

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7. REFERENCES

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8. APPENDIX

Table 8. List of phytochemicals in *Punica granatum* L.

SL. No.	Chemical compound	Molecular formula	Molecular weight (g/mol)
1.	(2e,6e)-9-(3,3-Dimethyl-2-Oxiranyl)- 3,7-Dimethyl-2,6-Nonadienyl Phenyl Sulfide	$C_{21}H_{30}OS$	330
2.	1,2,3,4,6-Penta-O- Galloyl-Beta-D- Glucose	$C_{41}H_{32}O_{26}$	940.681
3.	1,2,3-Tri-O-Galloyl- Beta-4c-1- Glucopyranose	$C_{27}H_{24}O_{18}$	636.469
4.	1,2,3-Tri-O-Galloyl- Beta-4c1-Glucose	$C_{27}H_{24}O_{18}$	636.4687
5.	1,2,3-Tri-O-Galloyl- Beta-D-Glucose	$C_{27}H_{24}O_{18}$	636.4687
6.	1,2,4,6-Tetra-O-Galloyl-Beta-D-Glucose	$C_{34}H_{28}O_{22}$	788.576
7.	17-Alpha estradiol	$C_{18}H_{24}O_2$	272.38196
8.	17-Beta estradiol	$C_{18}H_{24}O_2$	272.38200
9.	17-Beta estriol	$C_{24}H_{32}O_9$	464.5055
10.	1-Methylhexyl Acetate	$C_9H_{18}O_2$	158.241
11.	2 -Hydroxy-3-Methyl-4-Pyrone	$C_6H_6O_3$	126.11
12.	2 –Hydroxyacetylfuran	$C_6H_6O_3$	126.111
13.	2-(2-Propenyl)-Delta'- Piperideine	$C_8H_{13}N$	123.1955
14.	2,3- Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-one	$C_7H_{10}O_4$	158.1519
15.	2,3,4,5-Tetrahydro-6- Propenyl-pyridine	$C_8H_{13}N$	123.199
16.	2-Hydroxycyclopent-2-en-1-one	$C_5H_6O_2$	98.101
17.	2-Hydroxycyclopentadecanone	$C_{15}H_{28}O_2$	240.387
18.	2-O-Galloylpunicalin	$C_{41}H_{26}O_{26}$	934.633
19.	2s, 3s, 4s-Trihydroxypentanoic acid	$C_5H_{10}O_5$	150.130
20.	3,3,4 -Tri-O- Methylellagic acid	$C_{17}H_{12}O_8$	344.27
21.	3,3'-Di-O-Methylellagic acid	$C_{23}H_{20}O_{13}$	504.4
22.	3,4,8,9,10- Pentahydroxydibenzo(B,D)-pyran-6-one	$C_{13}H_8O_7$	276.198
23.	4n-Propylresorcinol	$C_9H_{12}O_2$	152.193
24.	5-Hydroxymethyl furfural	$C_6H_6O_3$	126.111
25.	Alpha tocopherol	$C_{29}H_{50}O_2$	430.717
26.	Apigenin	$C_{15}H_{10}O_5$	270.24
27.	Apigenin-4'-o-beta- glucopyranoside	$C_{21}H_{20}O_{10}$	432.378
28.	Apigenin-7-o-glucoside	$C_{21}H_{20}O_{10}$	432.38
29.	Asiatic acid	$C_{30}H_{48}O_5$	488.709
30.	Beta sitosterol	$C_{29}H_{50}O$	414.718
31.	Betulic acid	$C_{30}H_{48}O_3$	456.711
32.	Brevifolin	$C_{12}H_8O_6$	248.19
33.	Brevifolin carboxylic acid	$C_{13}H_8O_8$	292.199
34.	Brevifolin-carboxylic- acid-10-monosulphate	$C_{13}H_7KO_{10}S$	394.25
35.	Caffiec acid	$C_9H_8O_4$	180.159
36.	Casaurinin	$C_{41}H_{28}O_{26}$	936.649
37.	Castalagin	$C_{41}H_{26}O_{26}$	934.633
38.	Casuariin	$C_{34}H_{24}O_{22}$	784.544
39.	Catechin	$C_{15}H_{14}O_6$	290.271

Table 8. Continued

40.	Chlorogenic acid	$C_{16}H_{18}O_9$	354.311
41.	Cis-dimethyl morpholine	$C_6H_{13}NO$	115.176
42.	Cis-oleic acid	$C_{18}H_{34}O_2$	282.468
43.	Coniine	$C_8H_{17}N$	127.231
44.	Corilagin	$C_{27}H_{22}O_{18}$	634.455
45.	Coumestrol	$C_{15}H_8O_5$	268.224
46.	Cyanidin	$C_{15}H_{11}O_6^+$	287.247
47.	Cyanidin-3,5- diglucoside	$C_{31}H_{33}O_{19}$	709.586
48.	Cyanidin-3-glucoside	$C_{21}H_{21}O_{11}$	449.388
49.	Cyanidin-3-rutinoside	$C_{27}H_{31}O_{15}$	595.53
50.	Cycloartenol acetate	$C_{32}H_{52}O_2$	468.75
51.	Cymene	$C_{10}H_{14}$	134.222
52.	Daucosterol	$C_{35}H_{60}O_6$	576.859
53.	Delphinidin	$C_{15}H_{11}ClO_7$	338.696
54.	Delta allose	$C_6H_{12}O_6$	180.156
55.	Eicosenoicacid	$C_{20}H_{38}O_2$	310.522
56.	Elaidic acid	$C_{18}H_{34}O_2$	282.468
57.	Ellagic acid	$C_{14}H_6O_8$	302.194
58.	Ellagitannin	$C_{44}H_{32}O_{27}$	992.713
59.	Epicatechin	$C_{15}H_{14}O_6$	290.271
60.	Epigallocatechin 3-gallate	$C_{22}H_{18}O_{11}$	458.375
61.	Eschweilenol C	$C_{20}H_{16}O_{12}$	448.336
62.	Esterone	$C_{18}H_{22}O_2$	270.372
63.	Estradiol	$C_{18}H_{24}O_2$	272.388
64.	Estriol	$C_{18}H_{24}O_3$	288.387
65.	Ethyl oleate	$C_{20}H_{38}O_2$	310.522
66.	Ethyl palmitate	$C_{18}H_{36}O_2$	284.484
67.	Ethyl-brevifolin carboxylate	$C_{15}H_{12}O_8$	320.253
68.	Ferulicacid	$C_{10}H_{10}O_4$	194.186
69.	Flavan-3-ol	$C_{15}H_{14}O_2$	226.275
70.	Flavogallol	$C_{21}H_8O_{12}$	452.283
71.	Friedelin	$C_{30}H_{50}O$	426.729
72.	Friedooleanan-3-one	$C_{30}H_{50}O$	426.7264
73.	Fumaricacid	$C_4H_4CuO_4$	179.618
74.	Gallagic acid	$C_{28}H_{10}O_{16}$	602.372
75.	Gallic acid	$C_7H_6O_5$	170.12
76.	Gallocatechin-(4,8)- catechin	$C_{30}H_{26}O_{13}$	594.5196
77.	Gamma-sitosterol	$C_{29}H_{50}O$	414.718
78.	Genistein	$C_{15}H_{10}O_5$	270.24
79.	Genistin	$C_{21}H_{20}O_{10}$	432.381
80.	Granatin A	$C_{34}H_{24}O_{22}$	784.5412
81.	Granatin B	$C_{41}H_{28}O_{27}$	952.6448
82.	Hydroxyl cinnamic acid	$C_9H_8O_3$	164.16
83.	Hygrine	$C_8H_{15}NO$	141.214
84.	Icariside-d1	$C_{19}H_{28}O_{10}$	416.4196
85.	Idzein	$C_{15}H_{10}O_4$	254.241
86.	Idzin	$C_{21}H_{20}O_9$	416.382

Table 8. Continued

87.	Isobutyl phthalate	$C_{16}H_{22}O_4$	278.348
88.	Isoquercetrin	$C_{21}H_{20}O_{12}$	464.379
89.	Kaempferol	$C_{15}H_{10}O_6$	286.239
90.	Kaempferol-3-o-glycoside	$C_{21}H_{20}O_{11}$	448.38
91.	Linoleic acid	$C_{18}H_{32}O_2$	280.452
92.	Lupenone	$C_{30}H_{48}O$	424.713
93.	Luteolin	$C_{15}H_{10}O_6$	286.239
94.	Luteolin-3'-o-beta-d- glucoside	$C_{21}H_{20}O_{11}$	448.3769
95.	Luteolin-4'-o-beta- glucopyranoside	$C_{21}H_{20}O_{11}$	448.37690
96.	Maslinic acid	$C_{30}H_{48}O_4$	472.71
97.	Melatonin	$C_{13}H_{16}N_2O_2$	232.283
98.	Methyl-isopelletierine	$C_9H_{17}NO$	155.241
99.	Myricetin	$C_{15}H_{10}O_8$	318.237
100.	Naringin	$C_{27}H_{32}O_{14}$	580.539
101.	Neo-chlorogenic-acid	$C_{16}H_{18}O_9$	354.311
102.	N-methylpelletierine	$C_9H_{17}NO$	155.241
103.	N-nitrosoazacyclononane	$C_8H_{16}N_2O$	156.229
104.	Norhygrine	$C_7H_{13}NO$	127.187
105.	O-coumarinic acid	$C_9H_8O_3$	164.16
106.	Oleanolic acid	$C_{30}H_{48}O_3$	456.711
107.	Oleic acid	$C_{18}H_{34}O_2$	282.468
108.	Oxalic acid	$C_2H_2O_4$	90.034
109.	Oxandrolone	$C_{19}H_{30}O_3$	306.446
110.	Palmitic acid	$C_{16}H_{32}O_2$	256.43
111.	Para coumaric acid	$C_9H_8O_3$	164.16
112.	Para Menth-1-en-4-ol	$C_{10}H_{18}O$	154.253
113.	Pedunculagin	$C_{34}H_{24}O_{22}$	784.544
114.	Pelargonidin	$C_{15}H_{11}ClO_5$	306.698
115.	Pelletierine	$C_8H_{15}NO$	141.214
116.	Phenethyl-rutinoside	$C_{20}H_{30}O_{10}$	430.45
117.	Phlorizin	$C_{21}H_{24}O_{10}$	436.413
118.	Procyanidin b1	$C_{30}H_{26}O_{12}$	578.526
119.	Prodelphinidin b	$C_{30}H_{26}O_{14}$	610.524
120.	Prodelphinidin c	$C_{45}H_{38}O_{20}$	898.779
121.	Protocatechuic-acid	$C_7H_6O_4$	154.121
122.	Pseudopelletierine	$C_9H_{15}NO$	153.225
123.	Punicacortein-a	$C_{27}H_{22}O_{18}$	634.453
124.	Punicafolin	$C_{41}H_{30}O_{26}$	938.665
125.	Punicalin	$C_{34}H_{22}O_{22}$	782.528
126.	Punicic acid	$C_{18}H_{30}O_2$	278.436
127.	Punigluconin	$C_{34}H_{26}O_{23}$	802.559
128.	Pyrogallol	$C_6H_6O_3$	126.111
129.	Quercetin	$C_{15}H_{10}O_7$	302.238
130.	Quercetin-3-o- rutinoside	$C_{27}H_{30}O_{16}$	610.521
131.	Quercimeritrin	$C_{21}H_{20}O_{12}$	464.379
132.	Rutin	$C_{27}H_{30}O_{16}$	610.521
133.	Sedridine	$C_8H_{17}NO$	143.23

Table 8. Continued

134.	Serotonin	$C_{10}H_{12}N_2O$	176.219
135.	Sorbitol	$C_6H_{14}O_6$	182.172
136.	Stearic acid	$C_{18}H_{36}O_2$	284.484
137.	Stigmasterol	$C_{29}H_{48}O$	412.702
138.	Strictinin	$C_{27}H_{22}O_{18}$	634.455
139.	Succinic acid	$C_4H_6O_4$	118.088
140.	Tartaric acid	$C_4H_6O_6$	150.086
141.	Tellimagrandin I	$C_{34}H_{26}O_{22}$	786.558
142.	Tercatain	$C_{34}H_{26}O_{22}$	786.557
143.	Tricetin	$C_{15}H_{10}O_7$	302.238
144.	Tricin	$C_{17}H_{14}O_7$	330.292
145.	Tri-o-punicylglycerol	$C_{37}H_{92}0_6$	873.34
146.	Ursolic acid	$C_{30}H_{48}O_3$	456.711
147.	Valoneic acid	$C_{21}H_{10}O_{13}$	470.298

Table 9. List of phytochemicals in *Syzygium cumini* L.

SL. No.	Chemical compound	Molecular formula	Molecular weight (g/mol)
1.	1-Galloyl glucose	$C_{13}H_{16}O_{10}$	332.2601
2.	2-Phenylpropanal	$C_9H_{10}O$	134.178
3.	3,3', 4-Tri–o–methyl ellagic acid	$C_{17}H_{12}O_8$	344.275
4.	3,3'- Di–o–methyl ellagic acid	$C_{16}H_{10}O_8$	330.248
5.	3,5,7,4-Tetrahydroxy flavanone	$C_{15}H_{12}O_6$	288.255
6.	3,6- Hexahydroxydiphenoyl glucose	$C_{20}H_{18}O_{14}$	482.3485
7.	Acetyl oleanolic acid	$C_{31}H_{50}O_3$	470.738
8.	Ascorbic acid	$C_6H_8O_6$	176.124
9.	Bergenins	$C_{14}H_{16}O_9$	328.273
10.	Betulinic acid	$C_{30}H_{48}O_3$	456.711
11.	Bornyl acetate	$C_{12}H_{20}O_2$	196.29
12.	Caffeic acid	$C_9H_8O_4$	180.159
13.	Calacorene	$C_{15}H_{20}$	200.325
14.	Camphene	$C_{10}H_{16}$	136.238
15.	Catechin	$C_{15}H_{14}O_6$	290.271
16.	Chlorogenic acid	$C_{16}H_{18}O_9$	354.311
17.	Cineole	$C_{10}H_{18}O$	154.253
18.	Cis- farnesol	$C_{15}H_{26}O$	222.372
19.	Cis- ocimene	$C_{10}H_{16}$	136.238
20.	Citic acid	$C_6H_8O_7$	192.123
21.	Citronellol	$C_{10}H_{20}O$	156.269
22.	Corilagin	$C_{27}H_{22}O_{18}$	634.455
23.	Cyanidin	$C_{15}H_{11}O_6$	287.247
24.	Delfinidin	$C_{15}H_9O_7$	301.23
25.	Delphinidin-3gentiobioside	$C_{27}H_{31}O_{17}$	627.528
26.	Dihydrocarvyl acetate	$C_{12}H_{20}O_2$	196.29
27.	Dihydromyricetin	$C_{15}H_{12}O_8$	320.253
28.	Ellagic acid	$C_{14}H_6O_8$	302.194

Table 9. Continued

29.	Epifriedelanol	$C_{30}H_{52}O$	428.745
30.	Epi-friedlanol	$C_{30}H_{52}O$	428.745
31.	Esterase	$C_{21}H_{26}N_2O_3S$	386.51
32.	Eucarvone	$C_{10}H_{14}O$	150.221
33.	Eugenin	$C_{11}H_{10}O_4$	206.197
34.	Eugenol	$C_{10}H_{12}O_2$	164.204
35.	Ferulic acid	$C_{10}H_{10}O_4$	194.186
36.	Friedelanol	$C_{30}H_{52}O$	428.745
37.	Friedelin	$C_{30}H_{50}O$	426.729
38.	Gallic acid	$C_7H_6O_5$	170.12
39.	Geraniol	$C_{10}H_{18}O$	154.253
40.	Geranyl butyrate	$C_{14}H_{24}O_2$	224.3392
41.	Geranylacetone	$C_{13}H_{22}O$	194.318
42.	Hotrienol	$C_{10}H_{16}O$	152.237
43.	Isoquercetin	$C_{21}H_{20}O_{12}$	464.379
44.	Isorhamnetin 3-o-rutinoside	$C_{34}H_{42}O_{21}$	786.689
45.	Kaempferol	$C_{15}H_{10}O_6$	286.239
46.	Lauric acid	$C_{12}H_{24}O_2$	200.322
47.	Limonene	$C_{10}H_{16}$	136.238
48.	Linolenic acid	$C_{18}H_{30}O_2$	278.436
49.	Malvalic acid	$C_{18}H_{32}O_2$	280.452
50.	Malvidin	$C_{17}H_{15}O_7$	331.3
51.	Malvidin- 3- glucoside	$C_{23}H_{25}O_{12}$	493.4374
52.	Malvidin-3-laminaribioside	$C_{29}H_{35}O_{17}$	655.582
53.	Muurolol	$C_{15}H_{26}O$	222.372
54.	Myrcene	$C_{10}H_{16}$	136.238
55.	Myricetin	$C_{15}H_{10}O_8$	318.237
56.	Myricetin 3-o-4-acetyl-lrhamnopyranoside	$C_{23}H_{22}O_{13}$	506.413
57.	Myricetin-3-l-arabinoside	$C_{20}H_{18}O_{12}$	450.3497
58.	Myristic acid	$C_{14}H_{28}O_2$	228.376
59.	Myrtenal	$C_{10}H_{14}O$	150.218
60.	Myrtenol	$C_{10}H_{16}O$	152.233
61.	Nerol	$C_{10}H_{18}O$	154.253
62.	Oleanolic acid	$C_{30}H_{48}O_3$	456.711
63.	Oleic acid	$C_{18}H_{34}O_2$	282.468
64.	Palmitic acid	$C_{16}H_{32}O_2$	256.43
65.	Petunidin	$C_{16}H_{13}O_7$	317.273
66.	Petunidin- 3 - gentiobioside	$C_{28}H_{33}O_{17}$	641.555
67.	Pinocarveol	$C_{10}H_{16}O$	152.237
68.	Pinocarvone	$C_{10}H_{14}O$	150.221
69.	Quercetin	$C_{15}H_{10}O_7$	302.238
70.	Quercetin-3-d-galactoside	$C_{21}H_{20}O_{12}$	464.379
71.	Raffinose	$C_{18}H_{32}O_{16}$	504.438
72.	Rutin	$C_{27}H_{30}O_{16}$	610.521
73.	Stearic acid	$C_{18}H_{36}O_2$	284.484
74.	Sterculic acid	$C_{19}H_{34}O_2$	294.479
75.	Terpinolene	$C_{10}H_{16}$	136.238

Table 9. Continued

76.	Terpinyl valerate	$C_{15}H_{26}O_2$	238.3657
77.	Trans- Ocimene	C10H16	136.234
78.	Trigalloylglucose	$C_{27}H_{24}O_{18}$	636.471
79.	Vernolic acid	$C_{18}H_{32}O_3$	296.451
80.	Alpha Humulene	$C_{15}H_{24}$	204.357
81.	Alpha Copaene	$C_{15}H_{24}$	204.357
82.	Alpha Santalol	$C_{15}H_{24}O$	220.356
83.	Alpha Cadinol	$C_{15}H_{26}O$	222.372
84.	Alpha Pinene	$C_{10}H_{16}$	136.238
85.	Alpha Terpineol	$C_{10}H_{18}O$	154.253
86.	Beta Caryophyllene	$C_{15}H_{24}$	204.357
87.	Beta Myrcene	$C_{10}H_{16}$	136.238
88.	Beta Selinene	$C_{15}H_{24}$	204.357
89.	Beta Bitosterol	$C_{29}H_{50}O$	414.718
90.	Beta Phenylethanol	$C_8H_{10}O$	122.167
91.	Beta Pinene	$C_{10}H_{16}$	136.238
92.	Beta Terpinene	$C_{10}H_{16}$	136.238
93.	Gamma terpinene	$C_{10}H_{16}$	136.238
94.	Gamma cadinene	$C_{15}H_{24}$	204.357
95.	Delta cadinene	$C_{15}H_{24}$	204.357

Table 10. List of phytochemicals in *Mangifera indica* L.

SL. No.	Chemical compound	Molecular formula	Molecular weight (g/mol)
1.	(E)-Beta ocimene	$C_{10}H_{16}$	136.238
2.	(Z)-Beta ocimene	$C_{10}H_{16}$	136.238
3.	(Z,e)-Alpha farnesene	$C_{15}H_{24}$	204.357
4.	1, 9 diphenyl nonane	$C_{21}H_{28}$	280.452
5.	1-epi-cubenol	$C_{15}H_{26}O$	222.372
6.	2-furanometanol	$C_5H_6O_2$	98.101
7.	2-heptadecanone	$C_{17}H_{34}O$	254.458
8.	2-hydroxyacetophenone	$C_8H_8O_2$	136.15
9.	2-octene	C_8H_{16}	112.216
10.	4- o-methylgallic acid	$C_8H_8O_5$	184.147
11.	4,5-di-epi-aristolochene	$C_{15}H_{24}$	204.357
12.	7-epi-a-selinene	$C_{15}H_{24}$	204.357
13.	Alpha cadinol	$C_{15}H_{26}O$	222.372
14.	Alpha copaene	$C_{15}H_{24}$	204.357
15.	Alpha gurjunene	$C_{15}H_{24}$	204.357
16.	Alpha humulene	$C_{15}H_{24}$	204.357
17.	Allo-aromadendrene	$C_{15}H_{24}$	204.357
18.	Ambolic-acid	$C_{31}H_{50}O_3$	470.738
19.	Ambonic-acid	$C_{31}H_{48}O_3$	468.722
20.	Alpha phellandren-8-ol	$C_{10}H_{16}$	152.233
21.	Alpha phellandrene	$C_{10}H_{16}$	136.238
22.	Alpha pinene	$C_{10}H_{16}$	136.238

Table 10. Continued

23.	Aromadendrene	$C_{15}H_{24}$	204.357
24.	Alpha selinene	$C_{15}H_{24}$	204.357
25.	Alpha terpinene	$C_{10}H_{16}$	136.238
26.	Beta caryophyllene	$C_{15}H_{24}$	204.357
27.	Beta chamigrene	$C_{15}H_{24}$	204.357
28.	Beta elemene	$C_{15}H_{24}$	204.357
29.	Beta pinene	$C_{10}H_{16}$	136.238
30.	Beta selinene	$C_{15}H_{24}$	204.357
31.	Camphene	$C_{10}H_{16}$	136.238
32.	Caryophyllene oxide	$C_{15}H_{24}O$	220.356
33.	Cis-a-bergamotene	$C_{15}H_{24}$	204.357
34.	Cyperene	$C_{15}H_{24}$	204.357
35.	Delta 3-carene	$C_{10}H_{16}$	136.238
36.	Delta cadinene	$C_{15}H_{24}$	204.357
37.	Delta elemene	$C_{15}H_{24}$	204.357
38.	Diethyl phthalate	$C_{12}H_{14}O_4$	222.24
39.	Dodecanoic acid butyl ester	$C_{16}H_{32}O_2$	256.43
40.	Ellagic acid	$C_{14}H_6O_8$	302.194
41.	Epicatechin-3-o-gallate	$C_{22}H_{18}O_{10}$	442.376
42.	Eremophyllene	$C_{15}H_{24}$	204.357
43.	Ethyl decanoate	$C_{12}H_{24}O_2$	200.322
44.	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284.484
45.	Ethyl octanoate	$C_{10}H_{20}O_2$	172.268
46.	Ethyl tetradecanoate	$C_{16}H_{32}O_2$	256.43
47.	Furfural	$C_5H_4O_2$	96.085
48.	Gallic acid	$C_7H_6O_5$	170.12
49.	Galloyl glucose	$C_{13}H_{16}O_{10}$	332.261
50.	Gamma elemene	$C_{15}H_{24}$	204.357
51.	Genistin	$C_{21}H_{20}O_{10}$	432.381
52.	Geraniol	$C_{10}H_{18}O$	154.253
53.	Germacrene B	$C_{15}H_{24}$	204.357
54.	Gamma gurjunene	$C_{15}H_{24}$	204.357
55.	Gamma terpinene	$C_{10}H_{16}$	136.238
56.	Guaiol	$C_{15}H_{26}O$	222.372
57.	Hexadecanol	$C_{16}H_{34}O$	242.447
58.	Hexagalloyl glucose	$C_{48}H_{38}O_{30}$	1094.802
59.	Hinesol	$C_{15}H_{26}O$	222.372
60.	Humuulene epoxide ii	$C_{15}H_{24}O$	220.356
61.	Hydroquinone	$C_6H_6O_2$	110.112
62.	Icosane	$C_{20}H_{42}$	282.556
63.	Iriflophenone-di-o-galloylglucose	$C_{33}H_{28}O_{18}$	712.569
64.	Isomangiferolic-acid	$C_{30}H_{48}O_3$	456.711
65.	Kaempferol-3-o-glucoside	$C_{21}H_{20}O_{11}$	448.38
66.	Ledol	$C_{15}H_{26}O$	222.372
67.	Limonene	$C_{10}H_{16}$	136.238
68.	Linolenic acid	$C_{18}H_{30}O_2$	278.436

Table 10. Continued

69.	Mangiferic-acid	$C_{18}H_{32}O_2$	280.452
70.	Mangiferin	$C_{19}H_{18}O_{11}$	422.342
71.	Mangiferol	$C_{19}H_{18}O_{11}$	422.342
72.	Mangiferolic-acid	$C_{30}H_{48}O_3$	456.711
73.	Mangiferonic-acid	$C_{30}H_{46}O_3$	454.695
74.	Meta digallic acid	$C_{14}H_{10}O_9$	322.225
75.	Methyl citrate	$C_7H_8O_7$	204.135
76.	Methyl gallate	$C_8H_8O_5$	184.147
77.	Myrcene	$C_{10}H_{16}$	136.238
78.	Myristic-acid	$C_{14}H_{28}O_2$	228.376
79.	Neryl-acetate	$C_{12}H_{20}O_2$	196.29
80.	O-catechol	$C_6H_6O_2$	110.112
81.	Octadecane	$C_{18}H_{38}$	254.502
82.	Palmitic acid	$C_{16}H_{32}O_2$	256.43
83.	Palmitoleic acid	$C_{16}H_{30}O_2$	254.414
84.	Pantothenic-acid	$C_9H_{17}NO_5$	219.237
85.	Para coumaric-acid	$C_9H_8O_3$	164.16
86.	Para cymene	$C_{10}H_{14}$	134.222
87.	Pentagalloyl glucose	$C_{41}H_{32}O_{26}$	940.681
88.	Phytin	$C_6H_6Ca_5MgO_{24}P_6$	872.628
89.	Pogostol	$C_{15}H_{26}O$	222.372
90.	Protocatechuic acid	$C_7H_6O_4$	154.121
91.	Pyrogallol	$C_6H_6O_3$	126.111
92.	Quercetin	$C_{15}H_{10}O_7$	302.238
93.	Quercetin 3 arabinoside	$C_{20}H_{18}O_{11}$	434.353
94.	Quercetin 3-beta-d-glucoside	$C_{21}H_{20}O_{12}$	464.379
95.	Terpinolene	$C_{10}H_{16}$	136.238
96.	Tetracosyl- benzene	$C_{30}H_{54}$	414.762
97.	Tetragalloyl glucose	$C_{34}H_{28}O_{22}$	788.576
98.	Trigalloyl glucose	$C_{27}H_{24}O_{18}$	636.471
99.	Valencene	$C_{15}H_{24}$	204.357
100.	Viridiflorene	$C_{15}H_{24}$	204.357

Table 11. List of phytochemicals in *Tamarindus indica* L.

SL. No.	Chemical compounds	Molecular formula	Molecular weight (g/mol)
1.	1- Malic acid	$C_4H_6O_5$	134.087
2.	10-Octadecenoicacid	$C_{18}H_{34}O_2$	282.468
3.	1-Octanoate	C8H16O2	144.214
4.	2-Ethylthiazole	C_5H_7NS	113.178
5.	2-Methylthiazole	C_4H_5NS	99.151
6.	2-Phenyl acetaldehyde	C_8H_8O	120.151
7.	5-Hydroxymethyl furfural	$C_6H_6O_3$	126.111
8.	9-Decenoate	$C_{10}H_{17}O_2$	169.244
9.	Acetic acid	$C_2H_4O_2$	60.052
10.	Anthocyanin	$C_{15}H_{11}O$	207.252

Table 11. Continued

11.	Apigenin	$C_{15}H_{10}O_5$	270.24
12.	Benzyl benzoate	$C_{14}H_{12}O_2$	212.248
13.	B-sitosterol	$C_{29}H_{50}O$	414.718
14.	B-Carotene	$C_{40}H_{56}$	536.888
15.	Catechin	$C_{15}H_{14}O_6$	290.271
16.	Cinnamic acid	$C_9H_8O_2$	148.161
17.	Citric acid	$C_6H_8O_7$	192.123
18.	Compesterol	$C_{28}H_{48}O$	400.691
19.	Cycloartanol	$C_{30}H_{50}O$	426.729
20.	Eicosanoic acid	$C_{20}H_{40}O_2$	312.538
21.	Epicatechin	$C_{15}H_{14}O_6$	290.271
22.	Eriodictyol	$C_{15}H_{12}O_6$	288.255
23.	Ethyl-cinnamate	$C_{11}H_{12}O_2$	176.215
24.	Furfural	$C_5H_4O_2$	96.085
25.	Geraniol	$C_{10}H_{18}O$	154.253
26.	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43
27.	Iso-orientin	$C_{21}H_{20}O_{11}$	448.38
28.	Isovetexin	$C_{21}H_{20}O_{10}$	432.381
29.	L-(-)-Di-n-butyl maleate	$C_{12}H_{20}O_4$	228.288
30.	Limonene	$C_{10}H_{16}$	136.238
31.	Linalool	$C_{10}H_{18}O$	154.253
32.	Linoleic acid	$C_{18}H_{32}O_2$	280.452
33.	Linonene	$C_{10}H_{16}$	136.238
34.	Lupanone	$C_{30}H_{48}O$	424
35.	Lupeol	$C_{30}H_{50}O$	426.729
36.	Maleic acid	$C_4H_4O_4$	116.072
37.	Methyl-n-dotriacontanoate	$C_{33}H_{66}O_2$	494.889
38.	Methyl-n-Pentacosanoic	$C_{26}H_{52}O_2$	396.7
39.	Methyl-n-tricosanoate	$C_{24}H_{48}O_2$	368.646
40.	Methyl-salicylate	$C_8H_8O_3$	152.149
41.	Naringenin	$C_{15}H_{12}O_5$	272.256
42.	N-Docosanoate	$C_{22}H_{44}O_2$	340.592
43.	Nerol	$C_{10}H_{18}O$	154.253
44.	N-Heptadecanoate	$C_{17}H_{33}O_2$	269.449
45.	N-hexacosane	$C_{26}H_{54}$	366.718
46.	N-Hexadecanoate	$C_{16}H_{31}O_2$	255.422
47.	N-Nonacosanate	$C_{29}H_{58}O_2$	438.781
48.	N-Nonadecanoate	$C_{19}H_{38}O_2$	298.511
49.	N-Nonanoate	$C_9H_{18}O_2$	158.241
50.	N-Octadecanoate	$C_{18}H_{36}O_2$	284.484
51.	Nonacosatrienoic acid	$C_{29}H_{52}O_2$	432.733
52.	N-Tetradecanoate	$C_{14}H_{28}O_2$	228.376
53.	N-Tridecanoic acid	$C_{13}H_{26}O_2$	214.349
54.	Orientin	$C_{21}H_{20}O_{11}$	448.38
55.	Oxalic acid	$C_2H_2O_4$	90.034
56.	Pentadecatrienoate	$C_{16}H_{26}O_2$	250.382

Table 11. Continued

57.	Pinitol	$C_7H_{14}O_6$	194.183
58.	Procyanidin B2	$C_{30}H_{26}O_{12}$	578.526
59.	Procyanidin dimer	$C_{30}H_{26}O_{12}$	578.526
60.	Procyanidin trimer	$C_{45}H_{38}O_{18}$	866.781
61.	Safrole	$C_{10}H_{10}O_2$	162.188
62.	Succinic acid	$C_4H_6O_4$	118.088
63.	Tartaric acid	$C_4H_6O_6$	150.0868
64.	Taxifolin	$C_{15}H_{12}O_7$	304.254
65.	Trans-2-hexenal	$C_6H_{10}O$	98.145
66.	Vitexin	$C_{21}H_{20}O_{10}$	432.381
67.	A Carotene	$C_{40}H_{56}$	536.888
68.	Alpha pinene	$C_{10}H_{16}$	136.238
69.	Beta pinene	$C_{10}H_{16}$	136.238
70.	Beta amyrin	$C_{30}H_{50}O$	426.729
71.	Beta sitosterol	$C_{29}H_{50}O$	414.718
72.	2-acetylfuran	$C_6H_6O_2$	110.112
73.	2-Furancarboxaldehyde	$C_5H_4O_2$	96.085
74.	Cerotate	$C_{26}H_{51}O_2$	395.692
75.	Formic acid	CH_2O_2	46.025
76.	Lignocerate	$C_{24}H_{47}O_2$	367.638
77.	Luteolin	$C_{15}H_{10}O_6$	286.239
78.	Methyl-hexacosenoate	$C_{28}H_{56}O_2$	424.754
79.	Nicotinic acid	$C_6H_5NO_2$	123.111
80.	Octacosanylferulate	$C_{24}H_{38}O_4$	390.5561

Table 12. List of phytochemicals in *Phyllanthus emblica* L.

SL. No.	Chemical compound	Molecular formula	Molecular weight (g/mol)
1.	1,2,3,4,6-penta-Ogalloylglucose	$C_{41}H_{32}O_{26}$	940.681
2.	1,2-dihydroxyethylhydroxyfuran-2-one	$C_6H_8O_6$	176.124
3.	1,3,6-Trigalloyl glucose	$C_{27}H_{24}O_{18}$	636.469
4.	1,6-di O-galloyl-β-d-glucose	$C_{20}H_{20}O_{14}$	484.364
5.	1-Ogalloyl-beta-D-glucose	$C_{13}H_{16}O_{10}$	332.074
6.	3-ethylgallic acid	$C_9H_{12}O_5$	200.189
7.	5-hydroxymethylfurfural	$C_6H_6O_3$	126.111
8.	Amlaic acid	$C_{27}H_{24}O_{19}$	652.4681
9.	Apigenin7-O-glucopyranoside	$C_{21}H_{20}O_{10}$	432.381
10.	Arachidic acid	$C_{20}H_{40}O_2$	312.538
11.	Aspartic-acid	$C_4H_7NO_4$	133.103
12.	Astrgalin	$C_{21}H_{20}O_{11}$	448.38
13.	Behenic acid	$C_{22}H_{44}O_2$	340.592
14.	Beta sitosterol	$C_{29}H_{50}O$	414.718
15.	Betulin	$C_{30}H_{50}O_2$	442.728
16.	Betulinic acid	$C_{30}H_{48}O_3$	456.711
17.	Betulonic acid	$C_{30}H_{46}O_3$	454.695

Table 12. Continued

18.	Caffeic acid	$C_9H_8O_4$	180.159
19.	Chebulagic acid	$C_{41}H_{30}O_{27}$	954.664
20.	Chebulic acid	$C_{14}H_{12}O_{11}$	356.239
21.	Chebulinic acid	$C_{41}H_{32}O_{27}$	956.68
22.	Chlorogenic acid	$C_{16}H_{18}O_9$	354.311
23.	Corilagin	$C_{27}H_{22}O_{18}$	634.455
24.	Coumaric acid	$C_9H_8O_3$	164.16
25.	Daucosterol	$C_{35}H_{60}O_6$	576.859
26.	Emblicanin A	$C_{34}H_{22}O_{22}$	782.528
27.	Emblicanin B	$C_{34}H_{20}O_{22}$	780.512
28.	Friedelan-3-one	$C_{30}H_{50}O$	426.729
29.	Furosin	$C_{27}H_{22}O_{19}$	650.454
30.	Gallic acid	$C_7H_6O_5$	170.12
31.	Geraniin	$C_{41}H_{28}O_{27}$	952.648
32.	Glucogallin	$C_{13}H_{16}O_{10}$	332.261
33.	Isostrictinnin	$C_{27}H_{22}O_{18}$	634.455
34.	Kaempferol	$C_{15}H_{10}O_6$	286.239
35.	Kaempferol 3 O alpha L (6" methyl) rhamnopyranoside	$C_{21}H_{20}O_{10}$	432.381
36.	Leucodelphinidin	$C_{15}H_{14}O_8$	322.269
37.	Linoleic acid	$C_{18}H_{32}O_2$	280.452
38.	Linolenic acid	$C_{18}H_{30}O_2$	278.436
39.	Lupeol	$C_{30}H_{50}O$	426.729
40.	Lupeol acetate	$C_{32}H_{52}O_2$	468.766
41.	Luteolin 4'-O-neohesperidoside	$C_{27}H_{30}O_{15}$	594.522
42.	Luteolin-4'oneohesperiodoside	$C_{27}H_{30}O_{15}$	594.522
43.	Methyl gallate	$C_8H_8O_5$	184.147
44.	Myo-inositol	$C_6H_{12}O_6$	180.156
45.	Myricetin	$C_{15}H_{10}O_8$	318.237
46.	Myristic	$C_{14}H_{28}O_2$	228.376
47.	Myristic acid	$C_{14}H_{28}O_2$	228.376
48.	Oleanolic acid	$C_{30}H_{48}O_3$	456.711
49.	Pedunculagin	$C_{34}H_{24}O_{22}$	784.544
50.	Phyllaemblic acid B	$C_{15}H_{24}O_9$	348.348
51.	Phyllaemblic acid C	$C_{15}H_{24}O_8$	332.349
52.	Phyllaemblicin-A	$C_{27}H_{34}O_{14}$	582.555
53.	Phyllanemblinins A	$C_{27}H_{20}O_{17}$	616.44
54.	Phyllanemblinins B	$C_{27}H_{22}O_{18}$	634.455
55.	Phyllanemblinins C	$C_{41}H_{30}O_{28}$	970.663
56.	Phyllanemblinins D	$C_{27}H_{26}O_{20}$	670.485
57.	Phyllanemblinins E	$C_{27}H_{26}O_{20}$	670.485
58.	Phyllanemblinins F	$C_{27}H_{26}O_{20}$	670.485
59.	Phyllantidine	$C_{13}H_{15}NO_3$	233.267
60.	Phyllantine	$C_{14}H_{17}NO_3$	247.294
61.	Phyllemblin	$C_9H_{10}O_5$	198.174
62.	Proanthocyanidins	$C_{31}H_{28}O_{12}$	592.553
63.	Propyl 3,4,5-trihydroxybenzoate	$C_{10}H_{12}O_5$	212.201

Table 12. Continued

64.	Punigluconin	$C_{34}H_{26}O_{23}$	802.559
65.	Pyrogallol	$C_6H_6O_3$	126.111
66.	Quercetin	$C_{15}H_{10}O_7$	302.238
67.	Rutin	$C_{27}H_{30}O_{16}$	610.521
68.	Stigmasterol	$C_{29}H_{48}O$	412.702
69.	Triacontanoic acid	$C_{30}H_{60}O_2$	452.808
70.	Triacontanol	$C_{30}H_{62}O$	438.825
71.	Trihydroxysitosterol	$C_{38}H_{76}O_3S_3$	665.278
72.	Ursolic acid	$C_{30}H_{48}O_3$	456.711
73.	Zeatin	$C_{10}H_{13}N_5O$	219.248
74.	Zeatin riboside	$C_{15}H_{21}N_5O_5$	351.363
75.	Beta Amyrin ketone	$C_{30}H_{48}O$	424.713
76.	Beta Amyrin-3-palmitate	$C_{46}H_{80}O_2$	665.144
77.	Beta Carotene	$C_{40}H_{56}$	536.888
78.	Beta Humulene	$C_{15}H_{24}$	204.357
79.	Carpinusin	$C_{41}H_{30}O_{27}$	954.664
80.	Cinnamic acid	$C_9H_8O_2$	148.161
81.	Digallic-acid	$C_{14}H_{10}O_9$	322.225
82.	Dihydrokaempferol	$C_{15}H_{12}O_6$	288.255
83.	Ellagic acid	$C_{14}H_6O_8$	302.194
84.	Epigallocatechin	$C_{15}H_{14}O_7$	306.27
85.	Eriodictyol 7-O-glucoside	$C_{21}H_{22}O_{11}$	450.396
86.	Eriodictyol	$C_{15}H_{12}O_6$	288.255
87.	Galactaric acid	$C_6H_{10}O_8$	210.138
88.	Geranin A	$C_{30}H_{24}O_{10}$	544.512
89.	Hypophyllanthin	$C_{24}H_{30}O_7$	430.497
90.	Lupenone	$C_{30}H_{48}O$	424.713
91.	Naringenin	$C_{15}H_{12}O_5$	272.256
92.	Neochebulagic-acid	$C_{41}H_{30}O_{27}$	954.66
93.	Progallin-A	$C_9H_{10}O_5$	198.174
94.	Punicafolin	$C_{41}H_{30}O_{26}$	938.665

IN SILICO EVALUATION OF ANTI-COBRA VENOM ACTIVITY IN SELECTED FRUIT CROPS

By

GREESHMA A. S.

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Abstract of Thesis

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9. ABSTRACT

In the present investigation, a total of 522 phytochemicals from five plant species *viz. P. granatum*, *S. cumini*, *M. indica*, *T. indica* and *P. emblica* were selected as ligand molecules against 14 cobra venom toxic proteins such as PLA 2, CBT, LN 1, LN 2, LN 3, LN 4, LN 5, CA, CB, CYT 3, PRT, ACE, LAAO and SP and were docked using the tool AutoDock 4.2. The 3D structures of the target proteins were procured from databases or modeled structures procured from earlier authors and 519 phytochemicals procured from databases and remaining three molecules structures were created using the tool ChemSketch and its 3D structures were created in CORNIA. The docked structures with least binding energy, hydrogen bond and having hydrophobic interaction with critical residue were selected as the best lead molecule. The results showed that *Mangifera indica* can inhibit all the 14 cobra venom proteins. *P. granatum, P. emblica* and *T. indica* have no inhibitory compounds against CA and in case of *S. cumini* and *T. indica,* they don't have inhibitory compound against LN 2. In order to find out best lead molecule from these 522 phytomolecules against each venom protein without any error, top ranked five lead molecules were again docked using the tools Hex Server, PatchDock and iGEMDOCK and the results were subjected to DST analysis and selected the best lead molecules against nine proteins from *M. indicia,* two from *P. granatum*, two from *P. emblica* and one from *T. indica*. The best lead from *M. indicia* were 7-epi-a-selinene (-6.06 kcal/mol) on LN 1 and LN 3, mangiferolic acid on LN 4, ambolic acid on LN 5, 1-epi-cubenol on CA, isomangiferolic acid on CYT 3, mangiferonic acid on PRT and SP and mangiferolic acid on LAAO. The two best lead obtained from *P. granatum* were gamma sitosterol on PLA 2 and beta sitosterol on ACE. Similarly from *P. emblica* were betulonic acid on LN 2 and phyllantidine on CB and the only one best lead obtained from *T. indica* was procyanidin dimer on CBT. It was also noted that many lead molecules have multi-protein inhibitory activity. The overall results substantiated the traditional use of these plants as antidote to cobra venom. *In vitro* and *in vivo* experiments are to be essential for further confirmation.