"IDENTIFICATION OF LEAD COMPOUNDS WITH ANTI- COBRA VENOM ACTIVITY IN COMMON SPICES THROUGH *IN SILICO* METHODS"

By

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(2012-09-110)

Thesis

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DECLARATION

I hereby declare that this thesis entitled "**Identification of lead compounds with anti- cobra venom activity in common spices through *in silico* methods"** 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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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
3D	Three Dimensional
2D	Two dimensional
DST	Dempster-Shafer Theory
et al.	And others
HTS	High through put screening
Kcal	Kilocalorie
M	Molar
mL	Microlitre
mM	Millimolar
nM	Nanomolar
Da	Dalton
Mg	Milligram
NCBI	National Centre For Biotechnology Information
PDB	Protein Data Bank
WHO	World Health Organization
PLA2	Phospholipase A2
CBT	Cobrotoxin
CA	Cobramine A
CB	Cobramine B
CYT3	Cytotoxin 3
LAA	L- aminoacid oxidase
LN1	Long nuerotoxin 1
LN2	Long nuerotoxin 2
LN3	Long nuerotoxin 3
LN4	Long nuerotoxin 4
LN5	Long nuerotoxin 5
SER	Serine protease
PRO	Proteolase
ACE	Acetyl cholinesterase

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INTRODUCTION

1. INTRODUCTION

Snakebite and its consequence such as mortality and morbidity are global serious health concern wherever venomous snakes are present. Snakes are distributed all over the world except Polar Regions. However, majority of snakebites incidence occurred in rural areas especially in tropical and subtropical countries where the agriculture is the main occupation of the people. Over 3000 snake species have been reported from all over the world, of these, only 500 species are venomous and the annual death rate due to snake bite was estimated six times greater than the death due to tropical neglected diseases such as Dengue haemorrhagic fever, Cholera, Leishmaniasis, Schistosomiasis, Japanese encephalitis and Chagas disease (Williams *et al.*, 2010). In this circumstance, although it is not a pathogenic infectious disease, it is included along with other neglected tropical diseases in April, 2009 by World Health Organization (WHO, 2010). Most of the snake bite victims depend on traditional healers and they are not documenting properly the victim's treatment details. Therefore, it is difficult to estimate the actual snakebites death and morbidity rate. However, based on the data collected from hospitals, it was estimated that globally 5.0-5.5 million people are bitten by snakes resulting 40,000 amputations and 20,000 - 1,25,000 deaths per year and in India annual snakebite death rate is 15,000 to 50,000 (WHO, 2014).

Over 60 species of venomous snakes have been reported from India. However, the common four species distributed throughout India cause majority of the mortality, which are known as "Big four' species *viz Naja naja* L. (Indian cobra), *Daboia ruselli* Shaw & Nodder (Russell's viper), *Bungarus caeruleus* Scheider (Krait) and *Echis carinatus* Scheider (Saw scaled viper). Among these, Indian cobra cause high rate of mortality since its long curved sharp fangs can inject the venom into the vascular tissue and also the venom contains hyaluronidase which enhances spreading of the venom rapidly into the victim's blood stream.

Snake venom is a complex mixture of bio-molecules such as 90% proteins and 10% other molecules like nucleotide, inorganic ions, etc. Many of these

components have pharmaceutical importance and few proteins induce toxicity to the human body such as myotoxicity, cardiotoxicity, hemotoxicity and neurotoxicity etc. The venom composition is not always stable it may vary depend on species, age, geographical location etc.

In modern medicine anti-venom immunotherapy is the only specific treatment against snake venom. The major disadvantages of anti-venom therapy are (1) Side effects such as anaphylactic shock, pyrogen reaction and serum sickness. (2) Failure to neutralize the low molecular weight, less immunogenic toxic components of the venom will cause local hemorrhage, necrosis and tissue damage in snakebite victims. (3) Due to geographical variation in venom composition of snakes, anti-venom raised against the venom of a snake from a particular geographical origin may not be able to neutralize or prevent local effect of envenomation by snakes from other geographical locations. (4) Scarcity of sufficient amount of quality venom and lack of storage facility in rural areas. (5) Due to the difficulty in identifying the snake species, instead of using monovalent type, polyvalent type antivenom is commonly used, which may be hazardous to the patient and likely to be less effective.

Pharmaceutical companies are not at all interested to invest their fund for the discovery of therapeutics against snake venom mainly because they can't expect much profit since most of the victims are poor people in rural areas. Moreover, to find out a drug against a mixture of toxic proteins is not an easy task. Medicinal plants have been used against snakebites since time immemorial and recently it is well demonstrated that herbal medicine can effectively cure the disease caused by multifactorial causation like stomach problems and today physicians prescribing herbal medicines for such diseases. According to WHO 80% of the rural population still depend on traditional medicine for their primary health care. It is also believed that plant derived drugs induce less side effects than synthetic medicine and therefore, safe to use. Traditional medicines are evolved through the administration of various natural products by trial and error methods and envenomation is an ancient accidental health hazard. Several plant species have been suggested locally by the traditional healers against envenomation.

In India, about 350 plant species have been used against snakebites, however, its efficacy has seldom scientifically tested due to many reasons such as high investment, lack of raw materials, screening methodologies are time consuming and yielded less number of lead after high investment etc. Application of bioinformatics is the best option to overcome the forgoing problems and test the efficacy as well as the identification of the lead molecules present in the herbal extract for neutralizing venom toxicity. In the light of these, the present investigation was aimed to identify lead compounds with anti-cobra venom detoxification activity in common spices of Kerala *viz. Capsicum fruitescens* L., *Cinnamomum zeylanicum* Blume, *Piper nigrum* L. and *Allium cepa* through *in silico* methods.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 SNAKE BITE AND ITS PRESENT SCENARIO

Snake bites are well known accidental medical emergency in all over the world especially in rural areas. The burden of snake bite envenoming still has a great impact on the population and on health care system. The south-east asia region is one of the world's most affected regions, due to its high population density, widespread agriculture activities, presence of numerous venomous snakes. It is largely an occupational disease of food producers such as farmers, plantation workers, herdsmen, fishermen and also of wild life park rangers, military personnel, snake restaurant workers, snake handlers and collectors of snake skins. In 2004, WHO established a snakebite treatment group, whose role was to develop recommendations to reduce mortality according to international norms (Ghosh *et al.,* 2016). The WHO has included snake bite envenomation in the list of neglected tropical diseases in 2009 (Gupta and Peshin, 2014). One million envenomings and 75 000 deaths/year morbidity and mortality cases were reported in this region (WHO, 2016). So snakebite accounts for high morbidity and mortality than any other neglected tropical diseases.

Snake venom is highly modified saliva that is produced by special glands of certain species of snakes. The venom glands in elapids and viperids are situated behind the eye and are surrounded by compressor muscles. Venom glands are connected to fangs which are modified teeth. In elapids, the fangs are short and are mounted on a relatively fixed maxilla in front of the mouth. In viperids, the fangs are long and mounted on a rotatable maxilla, facilitating flat folding against the roof of the mouth. In humans, snakes usually inject venom subcutaneously or intramuscularly. The average dry weight of venom injected at a strike is approximately 60 mg (*N. naja*), 13 mg (*E. carinatus*) and 63 mg (*D. russelii*) respectively (Choudari *et al.,* 2014). Modern techniques of "venomics" (proteomics as applied to venoms) such as HPLC, SDS-PAGE, and mass spectrometry are revealing the enormous complexity of snake venoms (Warrell *et al.*, 2013). More than 20 types of toxic enzymes found in snake venom

throughout the world, out of these 12 are present in all types of snake venom (Raweerith, 2004). Snake venom is a combination of many different proteins, peptides , enzymes, substances with cytotoxic effects, neurotoxins, coagulants and anticoagulants and they are generally not dangerous when ingested. The major snake venom proteins include digestive hydrolases (proteinases, exopeptidase, endopeptidases, phosphodiesterases, metalloproteinases, and phospholipases), hyaluronidase (spreading fator). Most venoms contain l-amino acid oxidase, 5'-nucleotidase, DNAase, NAD-nucleosidase, phospholipase A2, and peptidases, serine proteases , Zinc metalloproteinases (WHO, 2016). Snake bite impact is varies with the venom complexity because upon species to species venom complexity is diverse.

There are three families of venomous snakes in south-east asia are elapidae, viperidae and colubridae (WHO, 2010). The concept of " big four" snakes considered as the most venomous snakes in these regions. These are Spectacled cobra (*N. naja*); common krait (*B. caeruleus*), Russell's viper (*D. russelii*), saw-scaled viper (*E. carinatus*), but other species, now recognized as being important, but they are not yet covered. So far 216 species of snakes have been identified in India of which 52 are known to be poisonous (Khan *et al.,* 2008).

2.1.1 **Snake bite treatments**

Some snake envenomation may not present with local signs thereby misleading the physician to think of other possibilities in the process allowing the golden hour to pass by. This is one of the main reasons of increased mortality attributed to krait bite in this part of north interior Karnataka (Khan *et al.,* 2008). Systemic diagnoses of treatment of snake bite depend on the various toxins and other compounds present in the venom (Reid, 1982). Neurotoxic symptoms can appear as early as 3 minutes after the bite but may be delayed by upto 19 hrs depending on the amount of venom injected along with other natural factors and host response (Ahmad *et al.,* 2008). Antisnake venom therapy is the only specific antidote to snake venom. It is introduced by Calmette in 1894. A most important

decision in the management of a snakebite victim is whether or not to give antivenom.

2.1.1.1. *Antisnake venom*

Antivenom is immunoglobulin [usually pepsin-refined F(ab')2 fragment of whole IgG] purified from the plasma of a horse, mule or donkey (equine) or sheep (ovine) that has been immunised with the venoms of one or more species of snake(Mohapathra and Mohantry, 2010). Antibodies raised against the venom of one species may have cross-neutralizing activity against other venoms, usually from closely related species. Bengal chemicals & pharmaceuticals, Calcutta Central Research Institute kasauli Haffkine Biopharmaceutical Company Ltd. Bombay. king's Institute of Preventive Medicine, Madras Serum Institute of India, Pune are the antisnake venom manufacturers in India (Martin, 2011).

2.1.1.2. *Limitations of Antisnake venom*

Usually more than 20% of cases will develop either early (within few hours) or late (5 days or more) allergic reactions following antivenom administration (Warrel, 2005). Snakes are different in different geographical area having different clinical manifestation for which different ASV is used (Mohapathra and Mohantry, 2010). Ignorance of species verification may adversely affect the body. Other limitations are the following.

1. Early anaphylactic reactions
2. Pyrogenic reactions
3. Late serum sickness Type reactions
4. High cost
5. Non-availability
6. Lack of storage facility in developing countries
7. Difficulty in identifying the snakes

Adminisration and the dosage of Antisnake venom therapy is well described by Khan, 2008., Mohapathra and Mohantry, 2010., WHO, 2016 . There is an urgent need to improve the design (species cover), quantity, and quality of

antivenoms produced in SouthEast Asia Region countries, in the interests of reducing snakebite mortality and morbidity.

2.2 PLANT AS A THERAPEUTIC AGENT

Throughout the human civilisation, man has searched for treatments to resist against minor and major ailments, mainly wounds and pain caused by insects, reptiles and other organisms. Man solely depend nature for his needs including food shelter and herbs for remedies against illness and to alleviate health conditions (Ravina, 2011). The medicinal value of plants has been recognized by almost every community in the world. In the nineteenth centuries, crude extracts of natural products particularly those derived from botanical species, provided the main source of folk medicines (Drews, 2000). In the latter part of the nineteenth century, bioactive compounds from plants began to be isolated in relatively pure form for medicinal use. For example, salicyclic acid, the precursor of aspirin, was isolated in 1874 from willow bark. More potent painkillers, such as morphine and codeine, were isolated from the opium poppy. The anti-malarial agent quinine was separated from cinchona (china bark) (Dias *et al.*, 2012). These discoveries enlightened the era of therapeutic drugs.

Before of the onset of synthetic era medical system is completely depend on the plants as a source of medicine. From that the plant derived drugs were enlighten and began with the isolation of morphine from opium from 19th century (Kinghorn, 2001). From that pharmaceutical research expanded with many other molecules derived from plants such as cocaine, codeine, digitoxin of which some are still in use. As a result they are investing large sums of money to try to find new plant chemicals that can be marketed as medicines. According to WHO statistics 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors (Rates, 2001). The advantage of natural drug from synthetic drug is that it has complex structures with tremendous oxygen content and abundant centres of stereochemistry (Jesse *et al.*, 2009). Almost one half of the chemical scaffolds from natural products cannot be

reproduced by synthetic chemistry. Plants have free radical scavenging molecules, including flavonoids, phenolics, anthocyanins and vitamins, which show antioxidant like activity (kumar, 2016). Graul *et al* reported that the drug Arteether is a potent medicine for malaria is derived from artemisinine a sesquiterpeine isolated from *Artemisia annua* (L.)(2001). Galanthamine is a natural product discovered from *Galanthus woronowii* Losinsk. In Russia. It is approved for the treatment of Alzheimer's disease (Heinrich and teoh, 2004: Pirttila *et al.,* 2004). The review "Medicinal plants: A source of new drug "by Aravind kumar shakhya. In this he listed the topmost plant derived drugs (2016). Human beings are in the race of endemic and epidemic diseases since ages and have been a potential hunter in search of medicines from the nature. Effectiveness, easy availability, low cost and comparatively being devoid of serum toxic effects are the benefits of herbal remedies.

A drug is a substance which is used in diagnosis or prevention of disease or as in the component of medication (FDA, 2015). The idea is that behind the drug action is the specific interaction with the biological macromolecule or the targets. The individual chemical molecules are required for the activity of drug. The single molecules were targeted for further development of drugs with the advancement of synthetic organic chemistry. It increases the momentum of drug developments. Synthetic sulfa drugs, the natural antibiotic penicillin, from *Penicillium notatum*, the semi-synthetic antibiotic tetracycline, produced from natural chlortetracycline elaborated by *Streptomyces aureofaciens* and the anti-tubercular aminoglycoside streptomycin, from *Streptomyces griseus* were all landmark discoveries of the 1930s and 1940s (CDD, 2013). The first synthetic drug, chloral hydrate, was discovered in 1869 and introduced as a sedative-hypnotic (Jones, 2011). Drug designing methods have been developed since the mid- twentieth century allowing a more specified approach to the discovery of novel agents. In the last couple of decades, genomics and proteomics have identified huge numbers of novel targets for future drug research. Structure based drug design involving X-ray crystallography and molecular modelling has been developed over the last 30 years allowing researchers to investigate how active

compounds interact with their targets at the molecular level. *Denovo* drug design has been developed over a similar period allowing scientists to study the active site of the target, then design novel structures *in silico* as potential lead compounds. Whether human-made or natural, the most important criteria for a medicine's use is safety, effectiveness and quality (Patrick *et al.,* 2013). Plants are best source for the identification of lead compounds. Because decoction of phytochemicals were identified in plants (Drews, 2000).

2.3 TRADITIONAL USE OF PLANT AS MEDICINE

Traditional medicine (also known as indigenous or folk medicine) comprises medical aspects of traditional knowledge that developed over generations within various societies before the era of modern medicine (WHO, 2000). In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. The WHO Traditional Medicine (TM) Strategy 2014–2023 was developed in response to the World Health Assembly resolution on traditional medicine. The goals of the strategy are to support member States in: 1. harnessing the potential contribution of TM to health, wellness and people centred health care; 2. promoting the safe and effective use of TM by regulating, researching and integrating TM products, practitioners and practice into health systems, where appropriate. The strategy aims to support Member States in developing proactive policies and implementing action plans that will strengthen the role of TM plays in keeping populations healthy. WHO's mission is to help save lives and improve health. (WHO, 2014).

Drugs take years to get through the research and development pipeline, at enormous cost, and rising drug resistance, partly caused by misuse of medicines, has rendered several antibiotics and other life-saving drugs ineffective. So scientists and pharmaceutical companies are increasingly searching TM for new drug sources. In India 70% of the population depend on traditional medicine to meet their health care needs. Many authors have reported the traditional use of plants aginst different ailments. The use of plants differes within different community, it will depend on their culture, availability, from the hereditary

resources. It has been listed in the papers put out by Muthu and Chelliah, 2006; Pan *et al.,* 2014; WHO, 2014.

2.4 ANTIDOTE PLANTS

From the time immemorial traditional medicine recommended many plants active against different effects of snakebite. India has an extensive tradition in the usage of medicinal plants. More than 100 plants that grow in India have been reported as snake venom antidotes (Kirtikar and Basu, 1975; Alam and Gomes, 2003). The indigenious peoples from different community follows their own distinct culture, food habit and they have rich knowledge of traditional medicine. Even today, with the use of advanced medical technologies certain local communities practise medicinal plants to cure several diseases, particularly used as folk medicine to treat snakebites (Makhija and Khamar, 2010). Molts (1992) stated that 578 species of higher plants from 94 families have been cited in the literature as being active against snakebite. Generally an aqueous, methanol or ethanol extract is prepared out of the plant parts. Topical application of the plant or its sap onto the bitten area, chewing leaves or barks or drinking plant extracts or decoctions or injecting the extracts are some procedures intended to counteract snake venom activity (Gomes *et al.,* 2010).

Several literatures suggested the usage of medicinal plants against snakebite (Martz 1992; Makhija and Khamar, 2010; Gomes *et al.*, 2010; Hasan *et al.,* 2016). Leaf paste of *Azadirachta indica* with rock salt is used against viper bites. *Aristolochia indica* is used as a decoction for snake bite (Gomes *et al.,*2010). The ethnomedicinal plant investigation among the traditional people revealed significant antivenom properties in plants such as *Eclipta prostrata* L. ,*Hemidesmus indicus* (L.) R. Br. , *Jatropha curcas* L. ,*Mimosa pudica* L. ,*Rauvolfia serpentine* (L.) Benth. , *Vitex negundo* L. , *Strychnos nux-vomica* L. , *Withania somnifera* (L.) Dunal (Naidu *et al.,* 2013). They mainly use leaf, root, bark, rhizome, stem, fruit, flower, leaf stalk, and whole plant as antidote against snakebite. The reported plants are administered as decoction, extracts, paste and juices (Hasan *et al.*, 2016). Although some knowledge has already been obtained

which proves that plant ingredients have pharmacological effects on pathogenesis after snakebite, Plant extracts represent an extremely rich source of pharmacologically active compounds and possess more than one biochemical/pharmacological property. Interaction of such compounds with the toxins/enzymes leads to the neutralization/inhibition of their activities. So plant remedies may be beneficial for the treatment of snakebite and may find alternative to antivenom serum (Makhija and Khamar, 2010). The plants have a potential for discovery of novel compounds with fewer side effects for treatment of snake envenomation and can become a source of new drug with the affordable price.

2.5 ANTIVENOM DRUG - *IN SILICO* APPROACH

Drug discovery and development is an interdisciplinary, expensive and time consuming process. *In silico* methods are few techniques with computational methods in which have significant potential to improve drug discovery and development process. These *in silico* methods include databases, quantitative structure-activity relationships, pharmacophores, homology models and other molecular modeling approaches, machine learning, data mining, network analysis tools and data analysis tools that use a computer. According to the BCG survey, *in silico* techniques save an average of $130 million and 0.8 years per drug (Seifert *et al.,* 2003). Because it helps in selecting only a potent lead molecule so can reduce failures in late stage clinical trials. History and evolution of *in silico* approaches are well described by Ekins, 2007. Virtual screening is the widely accepted *in silico* method in drug discovery processes. The process of scoring and ranking molecules in large chemical libraries according to their likelihood of having affinity for a certain target, is generally referred to as virtual screening (Oprea and Matter, 2004). Virtual screening requires either on bioactive ligands for the target of interest (ligand-based virtual screening) or on the target itself (target based virtual screening) and the Fragment based drug designing. Basic principles of FBDD include emphasis on high quality-controlled chemical libraries, biophysical validation of protein–ligand interactions, hit optimization aided by structural biology and hit assessment by ligand efficiency (Erlanson *et*

al., 2013; Hopkins *et al.,* 2014). Vemurafenib and Venetoclax are the approved drugs designed by the virtual screening method Fragment based drug designing. 28 drugs are under clinical trial research (Erlanson *et al.,* 2016).

Ligand based virtual screening strategies have conducted in many researches to identify the lead molecules as an aid for drug developments. Several developments in virtual screening studies are identification of lead compounds against human hepatitis B viral capsid protein in three medicinal plants through insilico methods(Subin *et al.,* 2016) and validation of Russell's viper venom detoxification activity of azadiracta indica through in silico methods (Deepa *et al.,*2016). They well described the strategies of ligand based virtual screening.

However, recently a few efforts in this line have been reported. For the discovery of antivenom drug, identification of lead molecules with potential interaction to venom proteins is the first stage in drug discovery process. Compared with traditional wet lab experiments, virtual screening is a more direct and rational drug discovery approach and has the advantage of low cost and effective screening (Nisha *et al.,* 2014). Nisha *et al* identified the lead molecules with cobra venom neutralizing activity in three Indian medicinal plants *viz. Vitex negundo* L., *Curcuma longa* L. and *Acorus calamus* L. through docking methods (2014).

In silico technologies helps in the early detection and the identification of molecule with several side effects and interaction with other drugs. But *in silico* technology alone cannot guarantee the novel safe and effective lead molecule. More realistically, It's a screening strategy and future success depend on the integration of new promising technologies in medicinal chemistry (Bharath *et al.,* 2011).

2.6 DOCKING

Recently, bioinformatics has advanced to the level that it allows almost accurate prediction of molecular interactions that hold together a protein and a ligand in the bound state. Computer-aided docking is an important tool for gaining understanding of the binding interactions between a ligand (small molecule) and its target receptor (enzyme) (Anderson, 2003; Schneider, 2010) and has emerged as a reliable, cost-effective and time-saving technique for the discovery of lead compounds (Walters *et al.,* 1998; Schneider and Bohm, 2002; Waszkowycz *et al.*, 2001). Docking is then used to predict the bound conformation and binding free energy of small molecules to the target. Single docking experiments are useful for exploring the function of the target, and virtual screening, where a large library of compounds are docked and ranked, may be used to identify new inhibitors for drug development (Forli *et al.,* 2016). In recent years, the virtual screening approach for docking small molecules into a known protein structure is a powerful tool for drug design and has become an integral part of the drug discovery process.

2.6.1 AutoDock 4.2

AutoDock is a suite of free open–source software for the computational docking and virtual screening of small molecules to macromolecular receptor. AutoDock combines binding free energy force field with a Lamarckian Genetic Algorithm, providing fast prediction of bound conformations with predicted free energies of association (Morris *et al.,* 1998). The new version of AutoDock is the AutoDock 4.2 incorporates explicit conformational modeling of specified sidechains in the receptor to address this problem. This capability also provides an effective method for the analysis of covalently-attached ligands (Morris *et al.,* 2009). In order to find the suitable conformational space available to a ligand around a protein, 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. Then, a probe atom is sequentially placed at each grid point, the interaction energy between the probe and the target is computed, and the value

is stored in the grid. This grid of energies may then be used as a lookup table during the docking simulation (Morris *et al.,* 2009). AutoDock tools are implemented in the object-oriented programming language Python and is build from reusable software components (Lutz *et al.,*1999; Sanner *et al.,* 2005). Computational docking and AutoDock is well reviewed by Morris *et al.,* the developers of Autodock suite (2009).

2.6.2 Analysis of the docked results

Docking poses generated by the docking programs can be analysed using different parameters. There are many different metrics out there for determining which docked poses for a given compound are "good". The energy estimation (epdb keyword) is used to calculate the energy of a ligand pose as found in a complex, such as in an X-ray crystallographic structure, without performing any searches. The coordinates of the ligand will not be changed, and grid maps will be used only to estimate the different energy contributions (Sebastian, 2014). Larger set of diverse protein-ligand complexes with known inhibiton constants were used in AutoDock 4.2. The inhibitory constant (Ki) is the concentration needed to reduce the activity of that enzyme by half. Visual examination of predicted binding geometries (docking poses) thereby contributes crucially to the further development of a lead compound either towards enhanced binding affinity. Visualization is crucial for structure based drug design, several tools have been developed to add visual support for the autodock suite (Seeliger and Groot, 2010). PyMOL is the most frequently used program for generating publication quality pictures of molecular structures and offers multiple advanced rendering options (Rajalekshmi *et al.,* 2013). Additionally it provides exceptional 3D viewing functionalities which can be very useful in structure-based drug design. The molecular interactions among active sites and the ligand compounds, binding site analysis can be analysed using pymol viewer.

2.7 TARGET COBRA VENOM PROTEINS

Snake venoms consist of a complex mixture of proteins, peptides, nucleosides and inorganic ions. Although they are not the only toxic principles present in these venoms, neurotoxins are mainly responsible for the lethality. The main constituents of cobra venom include PhospholipaseA2, Cobrotoxin, Long neurotoxin1, Long neurotoxin2, Long neurotoxin3, Long neurotoxin4, Long neurotoxin5, Acetylcholinesterase, aminooxidase, CobramineA, CobramineB, Cytotoxin3, Serine Protease and Proteolase (Sreekumar *et al.*, 2014). Three crystal forms of *Naja naja* PLA2 were discovered through random crystallization screening. The crystallization conditions for both of these novel crystal forms are Ca^{2+} free whereas previously reported conditions include Ca^{2+}. PLA2 is one of the major components in snake venom, which catalyzes the hydrolysis of fatty acid esters at a second position of 1,2di acyl-sn-phosphoglycerides requiring Ca++ ion as cofactor and have wide range of pharmacological activities such as neurotoxicity (pre and post synaptic), myotoxicity (local and systemic), cardiotoxicity, anticoagulant, convulsant, hypotensive, haemolytic, haemorrhagic, platelet aggregation and oedema inducing activity. HIS47 and ASP90 were marked as active residue of this enzyme. HIS47 is the preceding residue of ASP48 in which Ca^{2+} binds during catalytic reaction, hence, it was taken as active residue for docking. The two newly determined structures, generate an informative structural ensemble from which structural changes due to Ca^{2+}, which is required for catalysis, and the effect of crystal contacts on side-chain conformations and oligomeric association can be inferred. Both of the newly determined structures reveal a trimeric oligomer as observed in the tetragonal structure; this appears to be a unique feature of the *Naja naja* enzyme (Segelke *et al.*, 1998).

Cytotoxin A, also known as cobramine A having high cytotoxicity to Yoshida sarcoma and Ascites hepatoma cells, was isolated from the venom of the Indian cobra by column chromatography on Sephadex G-75, G-50 and CM-

cellulose. CytotoxinI contains 60 amino acid residues and contains no tryptophan, histidine, nor phenylalanine (Hayashi *et al.,* 1971). The amino acid sequence of Cytotoxin II (Cobramine B) was determined with the aid of tryptic digestion and cleavage by cyanogen bromide. Although their pharmacological actions are quite different, the amino acid sequences of cytotoxins were similar to those of cobra neurotoxins (Gasanov *et al.,* 2014).

The postsynaptic neurotoxins, named according to their site of action, bind to the nicotinic acetylcholine receptors in the plasma membrane of nerve and muscle cells and thereby prevent binding and the coupled depolarizing effect of acetylcholine. LN1 designated as Toxin A, isolated from the venom of the Indian cobra by column chromatography on Sephadex G-75, G-50 and carboxymethyl (CM)-cellulose, was homogeneous by the criteria of end group analysis and polyacrylamide gel electrophoresis. The complete amino acid sequence of the toxin which contains 71 amino acid residues (no methionine) was determined (Nakai, 1971). LNs are low molecular weight proteins having 70-74 amino acid residues crosslinked by disulfide bridges. They are classified as Long neurotoxin1, Long neurotoxin2, Long neurotoxin3, Long neurotoxin4 and Long neurotoxin5 (Walkinshaw *et al.*, 1979). The active sites were defined using the residues THR22, LYS23, TYR21, GLN55 and ARG33 for LN1, LN2, LN3, LN4 and LN5 respectively.

Snakevenom LAAOs (EC 1.4.3.2) are flavoenzymes belonging to the class of oxidoreductases that catalyze the stereospecific oxidative de-amination of L-amino acids. L- amino acid oxidases are usually homodimeric with cofactors FAD covalently linked to their chemical structure. The yellow colour of venoms rich in these enzymes is related to the presence of the pigment riboflavin present in the cofactors. Recent proteomic analyses of snake venoms show that metalloproteinases represent major components of venoms. In addition to hemorrhagic activity, members of the SVMP family also have fibrinolytic activity, act as prothrombin activators, activate blood coagulation factor X, possess apoptotic activity, inhibit platelet aggregation, are pro-inflammatory and

inactivate blood serine proteinase inhibitors. Clearly the SVMPs have multiple functions in addition to their well-known hemorrhagic activity (Markland and Swenson, 2012). Serine protease is another enzyme responsible for heamotoxicity. Serine protease belong to the clan PA family of S1 of trypsin like serine proteinase (Page and Di, 2008). Like trypsin, they cleave polypeptide chains on the c terminal side of positively charged aminoacid residues.

2.8 SPICES SELECTED FOR THE STUDY

A Spice is an aromatic or pungent vegetable substance used to flavour food, Kerala has its own treasure of spices which helps in the treatment of many diseases, in the production of cosmetics and perfumes or as a vegetable in the domestic applications. Spices not only add flavour to food, but are loaded with a number of phytochemicals. A wide range of chemical compounds including alkaloids, coumarins, flavonoids, benzofurans, terpenoids, steroids have been isolated from spices extracts and metabolites which have been found to posses various pharmacological, nematicidal and insecticidal activities (Santhosh *et al.,* 2010). A wide variety of phenolic substances derived from spice possess potent antimutagenic and anticarcinogenic activities and antiinflammatory activities (Surh, 2002). Docking studies against different therapeutic targets are also being taken up. The results of such studies showed that there is a number of promising lead compounds in spices yet to be discovered.

2.8.1 ***Piper nigrum*** **L.**

Piper nigrum (family Piperaceae) is a valuable medicinal plant. It is one of the most commonly used spices and considered as "The King of Spices'' among various spices. Hot and pungent peppercorns are obtained from black pepper which is the most famous and one of the commonly used spices throughout the world. It is widely used in different traditional systems of medicine like Ayurvedic and Unani System of medicines(Ahmad *et al.,*2012). Pharmacological activities of piper nigrum is well described by several authors. Piperine exhibits diverse pharmacological activities like antihypertensive and antiplatelets (Taqvi *et*

al.,2008), antioxidant, antitumor and antiasthmatic (Parganiha *et al.*, 2011), antipyretic, analgesic, antiinflammatory, antidiarrheal, antispasmodic, anxiolytic, antidepressants (Li *et al.*,2007), hepato-protective (Matsuda *et al.*,2008), immunomodulatory, antibacterial, antifungal, antithyroids, antiapoptotic, antimetastatic, antimutagenic, antispermatogenic, anticolon toxin, insecticidal and larvicidal activities. Piperine has been found to enhance the therapeutic efficacy of many drugs, vaccines and nutrients by increasing oral bioavailability by inhibiting various metabolising enzymes (wattanathorn *et al.*, 2007). The fruits of Piper nigrum are used to produce white and green peppers. Khan and Siddiqui in 2007 evaluated the antibacterial potential of aqueous decoction of Piper nigrum L. (black pepper). *Piper nigrum* is also used as a flavoring agent (Ahmad *et al.*, 2012).

Some ethnobotanicals have been confirmed to have snake venom neutralizing properties (Borges *et al.*, 2005). The snake venom neutralizing activity of piper nigrum is well reported by several authors. Tiwari and Pande described the usage of piper nigrum for the treatment of snakebite by the traditional people (2004). Pepper roasted in *ghee* is given orally to the snakebitten animal in South eastern part of Chamoli district, Uttaranchal, India. Application of a root paste of *kali haldi* (black turmeric) on the wounds of the snakebitten animal and touching of a red hot iron on the wounds quickly are the other practices(Tiwari and Pande, 2007). The articles revealed that black pepper possesses significant *in vitro* and *in vivo* pharmacological potential for the treatment of different ailments and diseases and found to be safe. Many original research articles on the pharmacological potential of *Piper nigrum* (Black Pepper) or ''Piperine'' had been published so far. Piperine has also been found to increase the absorption of many drugs and shown bioavailability enhancing activity of many drugs and nutrients.

2.8.2 *Allium cepa* L.

Onion is the one of the most important commercial widely used vegetable not only in India but also all over the world. It is the oldest cultivated crop and

the pungent edible bulb of the lily family considered as a food of exceptional value for flavoring and seasoning. The most important properties of onion embraces on antioxidant, anticancer, antimicrobial, asthma, cardiovascular compounds like sulfur, organosulfur, calcium and riboflavin from onions have a range of health benefits such as anticarcinogenic, antiplatelet, antithrombotic, antiasthmatic, antidiabetic, fibrinolytic and hypocholesterolemic properties and other various biological actions including antibiotic effects (Ashwini and Sathishkumar, 2014). The outstanding characteristic of onion is its pungency, which is due to a volatile oil known as allyl-propyl disulfide. Onion has been accepted as an important source of valuable phytonutrients as flavonoids, fructo-oligosaccharides, thio-sulphinates and other sulfur compounds (Rune *et al.,* 2007). Bakru reported that onion juice is an all round medicine for the most therapeutic purposes (2011). The *Allium cepa* exhibits Antimicrobial properties. It has been reported by Slimestade *et al.,* 2007; Briggs *et al.,* 2011.

The snake venom neutralizing property of *Allium cepa* is also studied by different authors in different communities. Shekhawat and Batra (2006) reported the usage of *Allium cepa* by the traditional people against snakebite. People of Keshavraipatan Tehsil of Bundi district, Rajasthan, India uses two teaspoonful bulb juice of the plant mixed with mustard oil and administers to expel poison by vomiting. Bheel community of central India uses bulb extract mixed with mustard oil (Kadel and Jain, 2008). Herbs owning antivenom serum activity need to be properly recognized (plant components/compound) and cultivated, and understanding must be disseminated well so that at least first aid treatments can be supplied to lessen mortality of snake bite (Sajon *et al.,* 2017).

2.8.3 *Cinnamomum zeylanicum* Blume.

Cinnamomum is one such genus which has been extensively used for the treatment of wide-array of disorders in various traditional systems. cinnamon, is used as a spice for cooking purposes across the world. In Ayurvedic medicine, cinnamon is being used for common cold, cough, diabetes, fever, flatulence, indigestion and sore throught. Cinnamon is being prescribed in traditional

Chinese medicine for cold, diarrhoea, asthma, as an appetiser; to strengthen the uterus and increase fertility in women. Maridass and Victor well reviewed the ethnobotanical use of *Cinnamomum* zeylanicum (2008). Pharmacological activities of cinnamon is studied by Kokate *et al.*,2003 and Nawale and Pujari, 2013.

It has been mentioned in certain literature that *Cinnamomum zeylanicum* was used for snake bite in ancient Indian medicinal folkfare (Gomes *et al.*, 2010). Muthu and his Colleagues reported the traditional usage of *cinnamomum zeylanicum* against snake bite in kanchipurm community (2006). Stem bark is the active ingredient in traditional medicine. *In vitro* evaluation of *cinnamomum zeylanicum* against *Naja Kauthia* snake is also studied by Mithul *et al.*, 2013.

2.8.4 *Capsicum frutescens* L.

Capsicum fruitescens was one of the spice plant products that have a rich source of antioxidants compounds(Faustino *et al.*, 2007). Capsaicinoid is phenolic compounds responsible for the pungency of the fruit that have been found mostly in capsicum fruits. The pungent compounds of *Capsicum frutescens* are capsaicin (69%), dihydrocapsaicin (22%), norhihydrocapsaicin (7%), homocapsaicin (1%) and homodihydrocpsaicin (1%) (Ezkeil, 2012). The availability of phytochemical compounds in this fruits indicate the important in choosing suitable foods that have rich source of antioxidant capacity as a prevention to the development of chronic disease such as diabetes and cancer (Rahim and Mat, 2012). Pharmacological activities of capsicum frutescens driven capsaicin are confirmed by Ezekiel (2012). Snakebite treatment using macerated fruits of *Capsicum frutescens* is reported by Gomes *et al.*, 2010.

MATERIALS AND METHODS

. 3. MATERIALS AND METHODS

The study on "Identification of lead compound with the anti-cobra venom activity in common spices through *in silico* methods" was conducted at Bioinformatics Centre, Saraswathy Thangavelu Extension Centre, JNTBGRI, Puthenthope during 2016-2017. Details of experimental materials used and the methodology followed in this work are presented in this chapter. Four spice plants and fourteen target cobra venom proteins were selected for the study.

3.1 SELECTION OF PLANTS

Four spice plants were selected based on the extensive literature survey, easy availability and wide usage as snake antidote by the traditional healers .

3.1.1 *Cinnamomum zeylanicum* Blume.

The plant is commonly known as true cinnamon tree, is a small evergreen tree belonging to the family Lauraceae, native to Sri Lanka. There are many literatutes showing the use of cinnamon bark as an active ingredient in the herbal treatment against snake venom. The bark is reddish brown and smooth. Nearly 128 phytochemicals were isolated so far from *Cinnamomum zeylanicum.*

3.1.2 *Piper nigrum* L.

Piper nigrum commonly known as the Black pepper is native to south India and is extensively cultivated there and elsewhere in tropical regions. *Piper nigrum* fruits and roots are used in the application of herbal snakebite remedies. About 213 phytochemicals were selected for docking.

3.1.3 *Capsicum frutescens* L.

Capsicum frutescens is a species of chilli pepper, commonly known as Birds eye chillies. The fruits are very pungent. The fruit and leaves are used in the snake bite treatment traditionally. One hundred and thirty one phytochemicals were selected as ligands for the present docking study.

3.1.4 *Allium cepa* L.

Allium cepa commonly called bulb onion, is a monocot bulbous perennial. Mainly the bulbous part of the plant mixed with adjuvant is used against snake bite traditionally. About 95 phytochemicals were selected as ligands for present docking study.

3.2 PREPARATION OF LIGAND MOLECULES

3.2.1 **Source of phytochemicals**

The details of phytochemicals screened in this study were obtained from the wide search of literatures. The finepoints were also collected from open source database is Dr.dukes phytochemical and ethnobotanical databases. Canonical SMILES were retrieved from different open access chemical databases such as Pubchem, Chemspider, fooDB. The 2D structure of the phytochemicals which are not available on open access databases were created using open access chemical structure drawing tool Chemsketch. Using SMILES notations the 3D structures of all phytochemicals generated in CORINA.

3.3 SELECTION OF TARGET PROTEINS

Fourteen toxic proteins were selected as target protein from *Naja naja* cobra venom. Among the fourteen Cobra venom proteins, structures of Phospholipase A2 (1A3D) and Cobrotoxin (1COD) were available in RCSB Protein Data Bank. The structures of remaining proteins were retrieved from Swiss Model repository. The 3D structures of nine proteins viz. Cobramine A (Swissprot ID P01447), Cobramine B (Swissprot ID P01440), Cytotoxin 3 (Swissprot ID P24780), Long neurotoxin1(Swissprot ID P25668), Longneurotoxin2 (Swissprot ID P25669), Longneurotoxin3 (Swissprot ID P25671), Longneurotoxin4 (Swissprot ID P25672), Longneurotoxin5 (Swissprot ID P25673) and Proteolase (Swissprot ID Q9PVK7) were retrieved from SWISSMODEL repository. Three Cobra venom proteins namely Serine protease (Swissprot ID: P86545) , L-amino acid oxidase (Swissprot ID A8QL58) and Acetyl cholinesterase (Swissprot ID

Q7LZG1) were modelled as per homology modeling using SWISSMODEL workspace.

3.4 DOCKING

There are different docking softwares to identify the preffered orientation of ligand protein complexes. Autodock 4.2, Hex Server, Patch dock etc. are some of the online docking softwares extensively used for the screening purpose. All selected phytochemicals from each plant were docked into the active site of 14 proteins using the open access docking software Autodock 4.2.

The active sites of the molecules were detected using the software applications Meta pocket, freely available online tool for binding site prediction. The docking was performed following the AutoDock procedure. This tool use Monte carlo Simulated Annealing and Lamarckian genetic algorithm for the generation of possible orientations of ligand at the active site of target proteins. The grid spacing was set to .375 A°.The grid box was set to centered the critical residues of each proteins by adjusting XYZ coordinates.The ligand bound protein complexes were analysed. The top ranked 5 molecules with least free energy of binding -5 Kcal/mol were selected as hit molecule .

3.4.1 **Post Docking Analysis**

Each hit molecule was analysed using free energy of binding, inhibition constant and Lipinskis rule of five. Lead molecule against each protein was identified from further analysis. To reduce error during lead selection, the top ranked 5 phytochemicals against each protein from all phytochemicals were again docked. The docking tools such as HEX server and Patchdock were used. The scores obtained were statistically analyzed following Dempster shafer theory and potential lead molecules were identified. The docked results were documented in a xls spreadsheet file format and uploaded on the website http://allamapparao.org/dst/application tool. The uploaded data were parsed and stored in 2D array and subsequently analyzed. The top-ranked molecules obtained through ranksum technique were selected.

3.4.2 **Mol inspiration property prediction tool**

To analyze the drug-likeness of the hit molecules for its property prediction, each molecule was submitted on open access molinspiration property prediction tool (www.molinspiration. com). The tool analyzes the molecular properties based on Lipinski's rule of five and violation in the particular property will be provided. The mol inspiration software was used to calculate MiLogP, total polar surface area (TPSA) and drug likeness. MiLogP (Octanol/water partition coefficient) is the parameter used to predict the permeability of the molecule across the cell membrane. Based on these parameters Drug likeness properties of the selected hits were analyzed and opted for leads.

Plate 1. Selected spice verieties. A. *Allium cepa* L., B. *Capsicum frutescens* L. C. *Piper nigrum* L., D. *Cinnamomum zeylanicum* Blume.

RESULTS

4. RESULTS

The main objective of the study was to identify lead compounds with anti-cobra venom detoxification activity in some common spices of Kerala namely *Cinnamomum zeylanicum* Blume., *Piper nigrum* L., *Allium cepa* L. and *Capsicum frutescens* L. through *insilico* methods. A total of 560 phytochemicals were collected from the spices selected for the study. Fourteen toxic proteins were selected as target cobra venom proteins for the screening of phytochemicals. Hits obtained against each target proteins and resulted lead molecules against them are discussed in detail.

4.1 DOCKED RESULT OF *ALLIUM CEPA* L.

Allium cepa, a herb of both medicinal and dietary importance is a rich source of many biologically active ingredients. Phytochemical analysis of the plant is well documented and a total number of 99 chemical compounds categorised to alkaloids, flavanoid, terpenes, steroids and resins so far reported from the plant. Out of the 99 molecules selected here as ligands against snake venom proteins, molecules having least free energy of binding (ΔG_{bind} <= -5 Kcal/ mol) selected as the hit molecules for further analysis to find the lead molecule. Five top hit molecules were selected from the list of compounds having free energy of binding in the order of lowest to higher ΔG bind.

About 13 molecules out of 99 phytochemicals showed the free energy of binding less than -5 Kcal/ mol against Acetyl cholineesterase. From which 31-Norlanostenol (-6.22), Alpha-amyrin (-6.22), Brassicasterol (-6.21), Campesterol (-5.98) and 4-alpha-methyl-zymosterol (-5.61) were selected as top hits. All hits except campesterol showed single hydrogen bond interaction with the bond type OHO. Brassicasterol and 31-Norlanoserol form hydrogen bond with the residue Phe 73. 4- alpha methyl zymosterol and Alpha – amyrin established hydrogen bond with the residues Ala104, Thr69 respectively. Among other hits brassicasterol has the least free energy (-6.21 Kcal/ mol) and less molecular weight, least inhibition constant, and the presence of more hydrophobic

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interaction than other hits. So Brassicasterol is selected here as the best lead molecule. It also promises the drug likeness properties according with the lipinki's rule of five.

Against Cobramine B, Triginollene is the only compound showed least free energy of binding (-5.33). It established 3 hydrogen bond interactions with active site residues Lys18, Lys12, Cys38 and it showed no violations in accordance with the lipinski's rule of five. It has positive value in druglikeness properties. Hence Triginollene is screened as lead against the target.

Analysis of *in silico* screening against Cobrotoxin resulted 5 hit molecules such as Alpha amyrin (-7.37), 31-Norlanostenol (6.82), 4-alpha methyl zymosterol (-6.68), 5-dehydro avenasterol (-6.34) and Campesterol (-6.11). Alpha amyrin and 31-Norlanostenol exhibits no hydrogen bonds. Rest of them established one hydrogen bond each. Among them 4 –alpha methyl zymosterol selected as lead molecule as it exhibits least free energy of binding and an O-H-O hydrogen bond with the residue Gly49. The bond length is 2.2 A° and is the lowest from others. Critical residue of the target is also involved in the hydrobhobic interaction. It exhibits one violation from Lipinski's riule of five but it showed high positive value in drug likeness properties.

Docking result of Cytotoxin screened Cycloartenol (-6.20), Alpha amyrin (-5.77), 31-Norcycloartenol(-5.73), 4-alpha methyl zymosterol (-5.67), Cycloeucalenol (-5.32) as hit molecues. Cycloartenol and 31-norcycloartenol established two hydrogen bond interactions each with the same residues Val41, Asp40 that are part of binding site residues. Cycloartenol has least free energy of binding but it has no hydrogen bond. So 31-norcycloartenol is selected as the best lead molecule because of the presence of 2 hydrogen bond and least inhibition constant and occurrence of significant hydrophobic interaction, eventhough it violate logP value in Lipinski's rule of five. It gave positive value in drug likeness properties.

Against L-amino acid oxidase 78 molecules showed free energy of binding less than -5 kcal/mol and selected Alpha sitosterol with ΔG_{bind} 10.80 Kcal/ mol formed two hydrogen bond with residues Gly88, Met62. 4 alphamethyl zymostenol with ΔG_{bind} 10.72 Kcal/ mol formed 2 hydrogen bond at the same residue Arg109. 5-dehydroavenasterol with ΔG_{bind} -10.19 Kcal/ mol formed a single hydrogen bond with the residue Arg109. Similarly 28-isofucosterol with ΔG_{bind} -9.64 Kcal/ mol also formed single hydrogen bond with the residue Arg109 and alpha amyrin withΔG_{bind} -10.21 Kcal/ mol has not established any hydrogen bonds were selected as the hit molecules. From these 4– alpha methyl zymostenol has taken as the lead molecule due to least inhibition constant and less molecular weight than Alpha sitisterol. It formed the hydrogen bond with the residue Arg109 , which is an active residue in the hydrophobic interactions. It also obeyed the lipinskis solubility and permeability rules.

Only two molecules, Cycloallin (5.10) and Melatonin (5.09) qualified as hit molecules with free energy of binding <= -5 Kacl/mol against Long nuerotoxin-1. Melatonnin established 2 hydrogen bonds with the residues Cys62, Pro71. Cycloallin showed the lesser ΔG_{bind} and established 3 H-bonds with active site residues. Considering the high binding affinity, Cycloallin is opted here as the lead molecule against the target. It completely obeys the lipinskis rule of five.

Against Long nuerotoxin-2, 3 molecules qualified as hit molecules. Alpha Amyrin (-5.31) established no hydrogen bond. Alpha sitosterol (-5.21) formed 2 hydrogen bond with the same residue Cys41. Brassicasterol (-5.19) also established 2 hydrogen bond at the same residue Cys41. Brassicasterol is selected here as the lead molecule as it is a low molecular weight compound which promises the drug likeness properties according to the lipinkis rule of five.

The compound Cycloallin (-5.21) alone showed ΔG_{bind} <= -5 Kcal/ mol against Long neurotoxin-3. It established 3 hydrogen bonds with active site residues and druglikeness properties of the molecule are also aceptable. So this molecule is considered as a lead molecule against the target venom protein.

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Five hits obtained against Long neurotoxin-4 are 31-Norcycloartenol (-5.58), Cycloeucalenol (-5.39), 4-Alpha methyl zymosterol (-5.29), Luteolin (-5.27) and Alpha sitosterol (-5.07). Alpha sitosterol is the highest molecular weight having compound which didn't establish H-bonds shown highest free energy of binging among the leads. 4-Alpha-Methyl-Zymosterol, 31-Norcycloartenol and Cycloeucalenol established 2 hydrogen bonds each at the same residues Pro64, Thr22. Luteolin established 4 hydrogen bonds at residues Pro64 ,Pro64, Thr22, Cys56 and the critical residue Gln 55 is also involved in the hydrophobic interaction. So Luteolin is considered here as the best lead because of lesser molecular weight and the promising bonding affinity and interaction with the target. It fully obeys lipinski's rule of druglikeness properties and shown no violations.

Against Long neurotoxin-5 16 compounds showed binding affinity with free energy of binding less than -5 Kcal/ mol. Of which Betasitosterol (-7.09), 4-Alpha Methyl Zymostenol (-5.98), Alpha-Amyrin (-5.75), 31-Norlanostenol (-5.48), 31-Norcycloartenol (-5.12), were screened as top hit molecules. 31-norcylo artenol, 31-norlanostenol and Alpha amyrin did not establish H-bonds. 4-alpha methyl zymostenol formed two H-bond bonds. Betasitosterol (-7.09) is the compound with least free energy of binding and established single H-bond. Arg 33 is the critical residue for the protein LN5 and is involved in the hydrophobic interaction with beta sitosterol. Considering the least free energy of binding, hydrogen bond interaction with critical residue at a promising bond length Betasitosterol is selected here as the best lead against the target. It also fully cooperated with Lipinski's rule of five.

PLA2 has moderate affinity on 82 molecules with free energy of binding less than or equal to -5Kcal/mol. Among them the compounds with least level of binding energy such as 31-Norlanostenol (-10.93), Brassicasterol (-10.52), Cycloartenol (-10.08), 24-Methylene-Cycloartenol (-9.20), 4-Alpha-Methyl-Zymostenol (-8.92) were ranked as top hits. Except Cycloartenol all of them formed a hydrogen bond each. 31-norlanostenol is selected as the lead with high

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binding affinity and has least inhibition constant (.00969) and least free energy of binding than other compounds. So it is selected as the lead compound. Cys44and His47 are the active site residues for PLA2. These residues involved in the hydrophobic interaction with 31- norlanostenol. It also obeys the drug likeness properties in accordance with the Lipinski's rule of five.

Against Proteolase 28 molecules exhibited free energy of binding less than -5 Kcal/mol. The compounds 4-Alpha-Methyl-Zymostenol (-7.17), Brassicasterol (-6.66), Campesterol (-6.57), Stigmast-7-En-3-beta-ol (-6.06 Kcal/ mol), Alliin (-6.00), were ranked as the top hit molecule. Allin and Stigmast-7-en-3-beta-ol shown single H-bond each with Glu465 and Cys474 respectively. 4-Alpha Methyl Zymostenol exhibited the least free energy of binding but it has no hydrogen bond interaction. The low molecular weight compound Allin is selected here as the best lead because of the presence of one hydrogen bond with the residue Glu465 which is one of active site residues of proteolase. The bond type is NHO which is stronger than OHO bonds with least bond length (2.4). The druggability characters of the lead are also in the acceptable range.

48 molecules showed free energy of binding lower than -5Kcal/mol against Serine protease. The compounds Caffeic acid (-6.58), Campesterol (-6.17), Protocatechuic acid (-5.72), Vanillic acid (-5.70) and 31-Norcycloartenol (-5.67) were screened as hit molecules. Among the hits caffeic acid exhibited least free energy of binding and inhibition constant with 4 OHO type and 2 OHN type H-bonds. Molecular weight of the compound is 180 g/mol and is a promising value for a drug candidate. Ser 31 is participated in the hydrophobic interactions, which is the critical residue for the protein serine protease. Considering the drugable characters of the target Caffeic acid is screened the best lead among the hits.

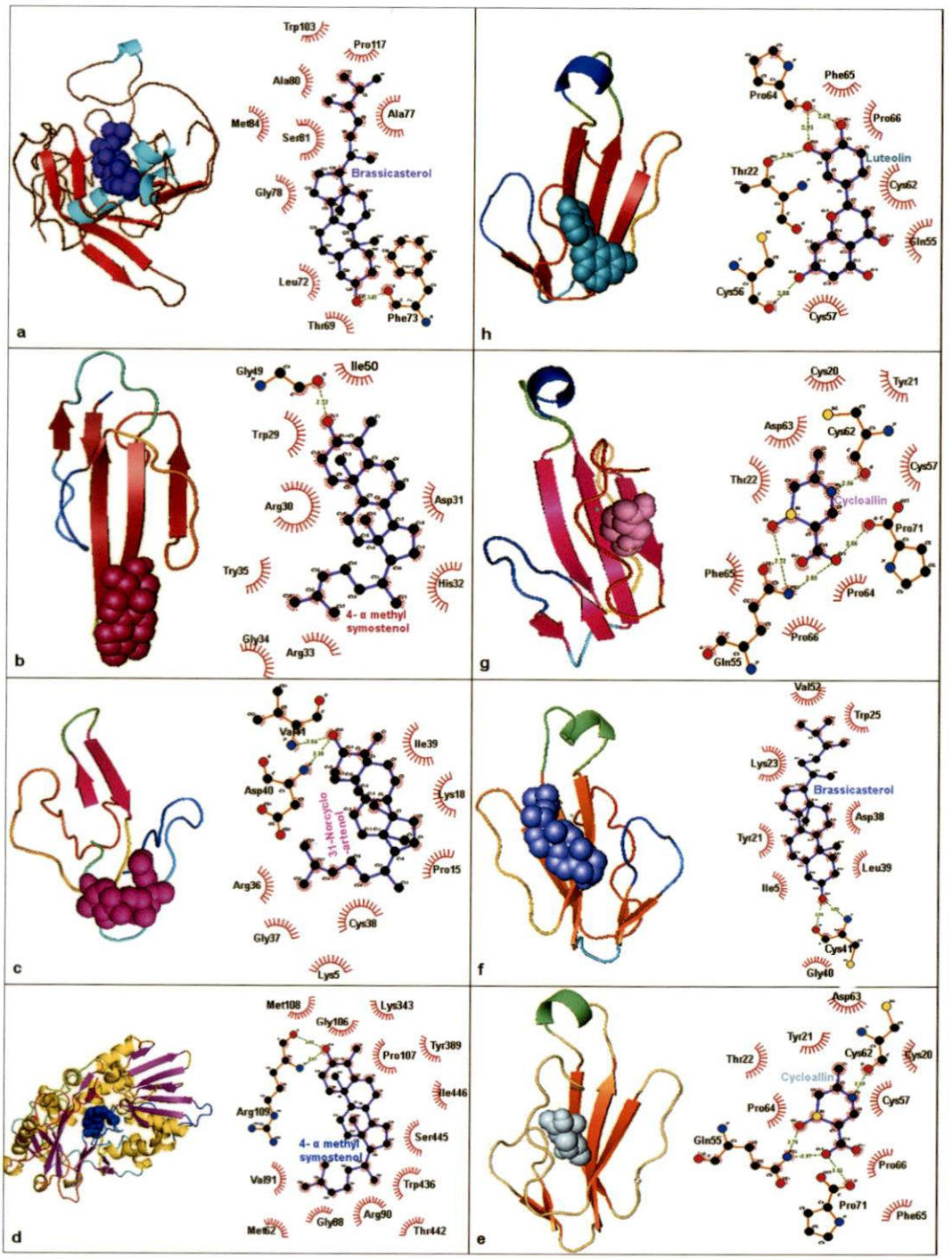

Figure 1: Docked poses of target and leads from *Allium cepa* in pymol viewer and ligplot.

a. ACE and Brassicasterol, b. CB and 4-Alpha methyl zymosterol, c. CYT and 31- Norcycloartenol, d. LAAO and 4- alpha methyl zymosterol e. LN1 and Cycloallin, f. LN2 Brassicasterol, g. LN3 and Cycloallin, h. LN4 and Luteolin

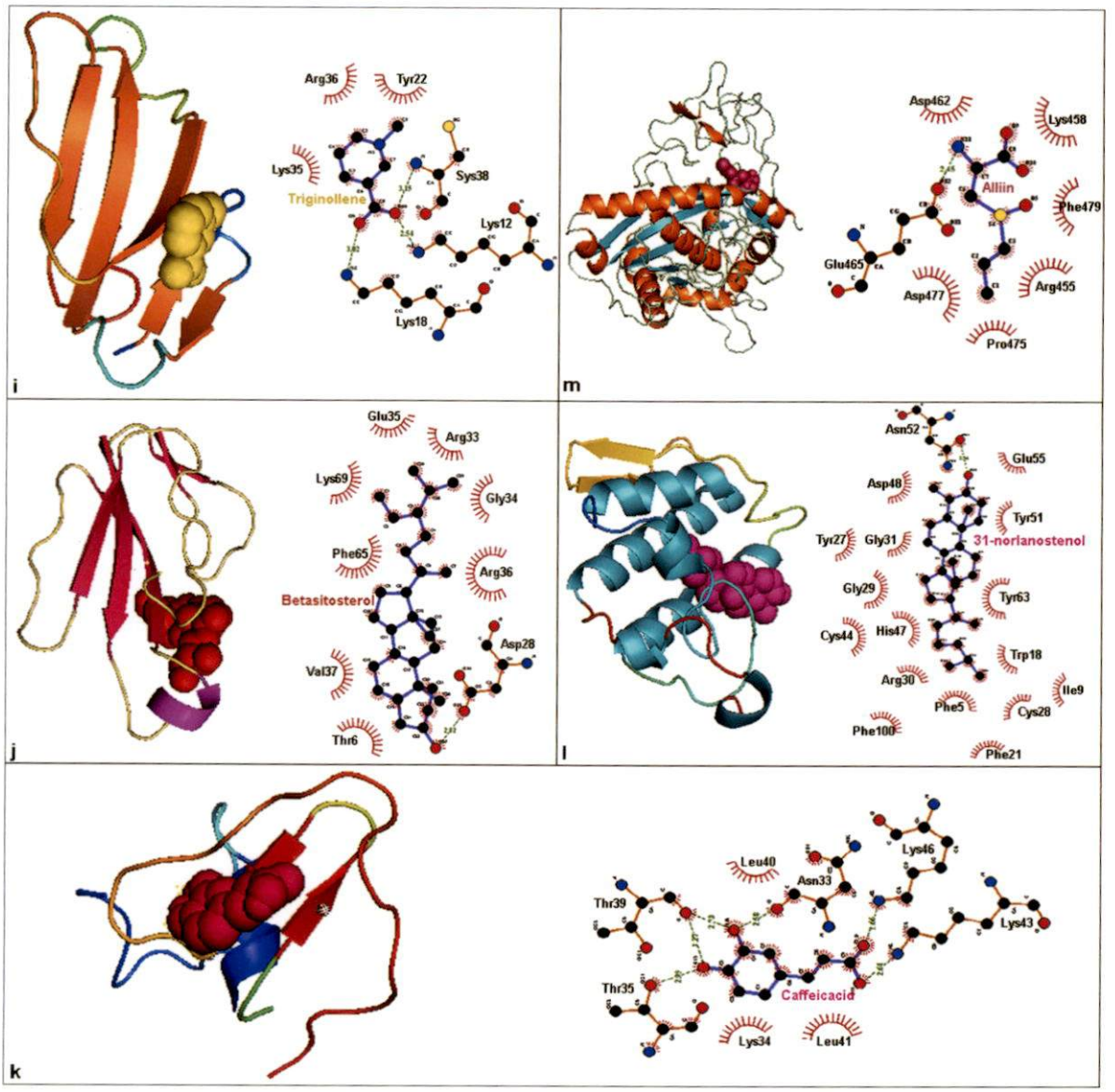

Figure 1. Continued.

i. CB and Trigonelline, j. LN5 and Beta Sitosterol, k. SER and Caffeic acid, l. PLA2 and 31- Norlanosterol, m. PRO and allin.

Table 1. Docked result of selected hits from *Allium cepa* against venom proteins.

Target proteins	Ligands	BE	IC(uM)	Hbond	Bond type	BL	Residue
Acetyl cholineesterase	4- alpha- ethyl - zymostenol	-5.61	77.70	1	OHO	2.70	Ala104
	31-Norlanostenol	-6.22	27.53	1	OHO	2.9	Phe73
	Alpha amyrin	- 6.22	27.54	1	OHO	2.9	Thr69
	Brassicasterol	**-6.21**	**27.84**	**1**	**OHO**	**2.63**	**Phe73**
	Campesterol	-5.98	41.23	0			
Cobramine B	Trigonelline						
Cobrotoxin	4-alpha methylsymostenol	-6.68	12.70	1	OHO	2.2	Gly49
	5-dehyroavenasterol	-6.34	22.69	1	OHO	3.1	Ser8
	31-Norlanostenol	-6.82	9.99	0			
	Alpha amyrin	-7.37	3.94	0			
	Campesterol	-6.11	33.49	1	OHO	2.5	Asn5
Cytotoxin	Cycloartenol	-6.20	28.33	0			
	Alpha amyrin	-5.77	58.80	0			
	31-Norcyclo-artenol	-5.73	62.78	2	OHN OHN	3.04 3.20	Val41 Asp40
	4-alpha methylsymostenol	-5.67	70.23	0			
	Cycloeucalenol	-5.32	124.95	2	OHN OHN	2.69 2.97	Val41 Asp40
Laminoacid oxidase	Alphasitosterol	-10.80	.1204	2	OHO OHN	3.0 3.2	Gly88 Met62
	4-alpha methylsymostenol	-10.72	.01385	2	OHO OHN	3.0 3.0	Arg109 Arg109
	Alpha amyrin	-10.21	.3289	0			
	5-dehyroavenasterol	-10.19	.03395	1	OHO	2.5	Arg109
	28- isofucosterol	-9.64	.08649	1	OHN	2.5	Arg109
LN2	Alpha amyrin	-5.31	129.08	0			
	Alpha sitosterol	-5.21	152.36	2	OHO OHN	2.21 3.12	Cys41
	Brassicasterol	-5.19	156.18	2	OHN OHO	1.59 1.91	Cys41
LN3	Cycloallin	-5.19	156.18	2	OHN OHO	1.59 1.91	Cys41
LN4	31-norcycloartenol	-5.58	80.63	2	OHO OHO	2.53 2.78	Pro64 Thr22
	Cycloeucalenol	-5.39	111.99	2	OHO OHO	2.69 2.53	Thr22 Pro64
	4-alphamethylzymostenol	-5.29	131.69	2	OHO OHO	2.53 2.36	Pro64 Thr22
	Luteolin	-5.27	136.78	4	OHO OHO OHO OHO	2.69 2.91 2.96 2.33	Pro64 Pro64 Thr22 Cys56
	Aapha sitosterol	-5.07	192.68	0			

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LN5	Betasitosterol	-7.09	6.37	1	OHO	2.8	Asp38
	4-alphamethylzymostenol	-5.98	224.49	2	OHO OHO	2.9 2.8	Val37 Val37
	Alphaamyrin	-5.75	60.57	0			
	31-Norlanostenol	-5.48	96.20	0			
	31-norcycloartenol	-5.12	176.37	0			
PLA2	31-Norlanostenol	-10.93	.00969	1	OHO	2.5	Asn52
	Brassicasterol	-10.52	.01933	1	OHO	2.4	Phe21
	Cycloartenol	-10.08	.04079	0			
	24-methylenecycloartenol	-9.20	.17899	1	OHO	1.5	Gly32
	Cycloeucalenol	-9.66	.08365	1	OHO	2.2	Gly31
Proteolase	4-alphamethylzymostenol	-7.17	5.52	0			
	Brassicasterol	-6.66	13.12	0			
	Campesterol	-6.57	15.39	0			
	Stigmast-7-en-3-beta-ol	-6.06	36.24	1	OHO	2.95	Cys474
	Alliin	-6.00	39.96	1	NHO	2.45	Glu465
Serine protease	Caffeic acid	-6.58	15.09	6	OHO OHO OHO OHO OHN OHN	2.99 3.27 2.73 2.58 2.66 2.68	Thr35 Thr39 Thr39 Asn33 Lys46 Lys43
	Campesterol	-6.17	29.79	2	OHO OHO	3.13 2.73	Gln190 Arg187
	Protocatechuic acid	-5.72	64.17	5	OHO OHO OHN OHO OHO	2.34 2.91 3.14 3.20 3.15	Thr39 Thr39 Lys46 Asn33 Leu41
	Vanillic acid	-5.70	66.05	4	OHO OHN OHO OHN	2.57 3.13 3.34 3.32	Thr39 Thr35 Asn33 Lys46
	31-Norcycloartenol	-5.67	69.34	2	OHO OHN	2.73 3.31	Thr42 Thr42

4.2 DOCKED RESULTS OF *PIPER NIGRUM* L.

Far reaching literature search and extensive review on the phytochemisty of the plant, information on so far reported 213 phytomolecules were collected and screened for lead optimisation against the venom of snakes particularly Indian Cobra. Hits obtained against each target proteins (table-2) and resulted lead molecules against them are discussed in detail.

About 50 molecules showed free energy of binding <=-5Kcal/mol against Acetylcholineesterase. Among them, compounds with lowest free energy of binding such as Beta cubebine (-6.61), Beta sitosterol (-6.19), campesterol -6.00), Cedrol (-5.91) and Epoxy dihydro caryophyllene (-5.88) were screened as top hits. Beta cubebine showed 4 H-bonds with residues Ala77, Asn129, Ser134, Gly136 followed by Beta sitosterol having a single H-bond at Lys130, Both Cedrol and Epoxy dihydro caryophyllene formed single hydrogen bond at the residues Glu75, Gly136 respectively. While campesterol established no H-bond interaction with the protein. Considering the least free energy of binding, lowest inhibition constant and presence of more H-bonds beta cubebine is selected here as the best lead molecule.

In Cobramine-A, 5 molecules were selected as top hits. They are 1-epi-cubenol (-5.70 Kcal/mol), Germacrene B (-5.59 Kcal/mol), Alpha gurjunene (-5.48 Kcal/mol), A-copaene (-5.14 Kcal/mol) and Caryophyllene oxide (-5.13 Kcal/mol). Among them 1-epi cubenol made single H-bonds at residue Tyr51. Epicubenol that exhibits the least free energy of binding, lowest inhibition constant and established an H-bond was selected here as the best lead molecule which is also concurred the Lipinski's rule of five.

Docking of Cobramine B determined that among the 17 phytomolecules having the free energy of binding <= -5Kcal/mol. Oxalicacid (-5.93), T-Muurolol (-5.8)Epicubenol (-5.56), Cubenol (-5.50) and Cubebol (-5.35) were selected as top hit molecules. Cubebol , Cubenol and Epicubenol showed 2 hydrogen bonds each at the same residues Arg36 and Asn60 followed by oxalic acid and T-Muurolol formed single hydrogen bonds each at the residues Lys12 and Pro30

respectively. Though oxalic acid is the least free energy of binding molecule, Cubenol with two H-bonds, considerable free energy of binding level, lower inhibition constant and acceptable solubility and permeability range of Lipinski's rule of five is considered here as the best lead against Cobramine B. Furthermore the critical residue, Lys23 is also showing hydrophobic interaction with the ligand.

Against Cobrotoxin, 16 molecules resulted ΔG_{bind} <= -5 Kcal/mol, of which Betasitosterol (-6.18), Campesterol (-6.11), 17-Betaestriol (-5.82), Cubebin (-5.38) and Trichostachine1 (5.31) with least free energy of binding were screened as top hits. Beta sitosterol showed no H-bond interaction. 17- Beta estriol and Campesterol formed single hydrogen bonds each at Asn 5, Cubebin is also formed an H-bond while Trichostachine established two H-bonds with Arg36 and Thr37 but it showed highest ΔG_{bind} among the hits. Considering the least free energy of binding level, lowest inhibition constant Campesterol was selected here as the best lead. It established one H-bond interaction with Asn 5, which is one of the crucial catalytically active residue for the target protein.

52 compounds against Cytotoxin 3 were shown ΔG_{bind} <= -5 Kcal/mol. Of them 5 molecules such as Stigmasterol (-6.77), Betasitosterol (-6.11), Campesterol (-6.11), Betacubebin (-6.09) and Retrofractamidea (-6.00) were selected as top hits. Particularly none of these hit molecules formed hydrogen bond with the target protein residues. However Stigmasterol is selected as the lead compounds with least free energy of binding and least inhibition constant (10 micromolar). It showed acceptable drug likeness properties while violates Lipinski's rule of five. Generally drug molecules evolved from natural sources may violate lipinski's rule (A. Ganesan, 2008).

Docking with L amino acid oxidase, resulted 186 active molecules with ΔG_{bind} <= -5Kcal/mol. Among them Campesterol, Betasitosterol, Stigmasterol, Betacubebin, Retrofractamide A and Quercitrin were selected as top hits. Though Campesterol (-10.07) had shown the least free energy of binding it didn't establish H-bond interacton with active site residues so it has been excluded from

the hit list. Among the hits Quercitin established 8 H-bonds but its free energy of binding and inhibition constant level is higher than the rest. Betacubebin and Retrofractamidea established 2 H-bonds each. Betasitosterol and Stigmasterol are the two hits with least free energy of binding -9.90 and -9.04 respectively. It is striking to note that the H- bonding residue, Arg90 of Stigmasterol is the critical residue in the active site of the target. So Stigmasterol was the best among hits and selected here as the best lead molecule as it showed one of the least free energy of binding, lower inhibition constant, acceptable Lipinski's rule of drug properties and H-bnond interaction with the critical residue Arg90.

In the case of Long Neurotoxin 1, about 57 compounds were showing binding affinity with ΔG_{bind} <= -5Kcal/mol . Out of them 1-Terpinen-5-ol (-6.00), Alpha-Selinene (-5.79), Piperitone (-5.82) and Alpha-Cubebene (-5.67) were considered as top hits. 1-Terpinen-5-ol formed single hydrogen bond at the residue Pro64 followed by Isochavinicacid showed two hydrogen bonds at the same residues Arg70 and hydrophobic interaction with the critical residue Thr22. It also fully obeyed the Lipinski's rule of five. Other two hits Alphacubebene and Alphaselinene has not established any hydrogen bonds. Considering the full acceptance of drug likeness properties according to Lipinski's rule, more H-bond interactions and hydrophobic interaction with the critical residue Isochavinic acid is selected as the best lead molecule.

Out of 62 active molecules against Long neurotoxin 2, five hits such as Beta Sitosterol (-7.09), Campesterol (-6.34), Beta Cubebine (-6.19), 17- Beta Estriol (-6.16) and (5,10,15)-Cadinen-4-Ol (-6.15), were considered for further analysis. Beta Cubebine established three hydrogen bonds at residues Val37, Lys69. Hydrogen bond interaction of (5,1015)-Cadinen-4-ol showed 2 H-bonds at the residue Val37, followed by 17- Beta Estriol formed one H-bond at Asp27, Beta Sitosterol and Campesterol established one hydrogen bond each at the residues Asp38 and Gly34 respectively. Among the hits Beta Cubebine showed no violation in the Lipinski's rule of five; established more H-bonds with the target and acceptable free energy of binding level, so qualified as the best lead.

About 62 active molecules were identified against Long neurotoxin 3. Of which 1-Terpinen-4-ol, Piperitone , Alphacubebene, Betaselinene and (1,8)-P-Menthadien-5-ol were considered as top hits. (1,89)-P-Menthadien-5-ol established single hydrogen bond at Pro64, followed by 1-Terpinen-4-ol exhibited 2 hydrgen bonds at residues Cys62 and Pro 64. There is no hydrogen bond interaction among other hits. Considering the lease free energy of binding level, lower inhibition constant and higher number of H-bonds 1-Terpinen-4-ol is selected as the best lead. It also compromises with the lipinskis permeability and solubility rules.

In the case of Long Neurotoxin 4, about 57 compounds were showing binding affinity with ΔG_{bind} <=-5Kcal/mol. Out of them 1-Terpinen-5-ol, Alpha-Cubebene, Alpha-Selinene and Piperitone were considered as top hits as their free energy of binding falls in the least level. 1-Terpinen-5-ol formed single hydrogen bond at the residue Pro64 followed by Isochavinicacid with two hydrogen bond at the same residues Arg70 while other hits showed no hydrogen bonds. Among them Isochavinic acid is selected as the best lead molecule as it established 2 hydrogen bonds and hydrophobic interaction with the active critical residue Gln55. There is no violations in the Lipinski's rule of five and acceptable range of drug likeness proproperties.

46 molecules were shown binding activity against Long neurotoxin-5 with ΔG_{bind} <=-5Kcal/mol. Out of which (5,1015)- Cadinen-4-ol, 17betaestriol, Betacubebine, Betasitosterol and Campesterol were the compounds with least free energy of binding and considered them as top hits. Hydrogen bond interaction of the hits revealed that (5,1015)-Cadinen-4-ol showed ΔG_{bind} -6.15 Kcal/mol with 2 hydrogen bond, followed by 17-betaestriol with ΔG_{bind}-6.16 Kcal/mol formed one hydrogen bond at the residues Asp27. Betacubebine (-6.19) established 3 hydrogen bonds at residues Val37and Lys69. Betasitosterol (-7.09) and Campesterol (-6.34) established single hydrogen bonds each at the residues Asp38 and Gly34 respectively. Considering the higher number of H-bonds, hydrophobic interaction of the critical residue, Arg33 with the ligand and the full

acceptance of Lipinski's rule of five beta cubebine is selected here as the best lead against LN5.

About 190 molecules showed free energy of binding <=-5Kcal/mol to -11.46 against PLA2. Among them, compounds with most least free energy of binding such as Betasitosterol, Campesterol, Guineensine, 6-Trans-piperamide-C-7-1 and Betacubebine were screened as top hits. Of the 5 hits 6-Trans-piperamide and Betasitosterol established no hydrogen bonds. Beta Cubebine showed 2 hydrogen bonds at residues Asn52, Tyr63 followed by Quineensine and Beta sitosterol formed one hydrogen bond each with residues Cys44 and Phe21respectively. Betacubebine is the best lead here as it established more H-bonds, hydrophobic interaction with the critical residue Asp48 and the drug likeness properties are in an acceptable range.

Against Proteolase, about 38 molecules shown binding affinity with ΔG_{bind} <= -5Kcal/mol. The compounds Beta Sitosterol, Cubebin, Campesterol, Piperine and Isochavicine were selected as the top hits. Cubebin having ΔG_{bind} -6.59 Kcal/mol showed 4 hydrogen bonds followed by Beta sitosterol having ΔG_{bind} -6.93 Kcal/mol formed 2 hydrogen bonds. Campesterol having the ΔG_{bind} -6.57 Kcal/mol has also formed two hydrogen bonds. Isochavicine and Piperine having higher ΔG_{bind} formed single hydrogen bonds at the same residue Glu440. Beta sitosterol was selected here as the best lead molecule as it exhibited lowest ΔG_{bind} and the lowest inhibition constant. It also obeyed Lipinski's solubility and permeability rules.

Compounds like Pcoumaricacid, Isochavinicacid, Caffeicacid, Cinnamicacid and Campesterol with most ΔG_{bind} were screened as top hits (tabel-23) from 33 active molecules (ΔG_{bind} <= -5Kcal/mol) against Serine protease. Caffeicacid established 6 H-bonds followed by Pcoumaricacid Cinnamicacid and Campesterol formed 2 hydrogen bonds each while Isochavinicacid formed single H-bond. Though Pcoaricacid is the compound with least free energy of binding it established lesser number of H-bonds when compared to Caffeicacid. Considering the nearest least free energy of binding and inhibition constant to the

top hit, appreciable number of H-bonds (6nos.) of both OHO and OHN bond type and the complete acceptance of Lipinski's rule of five, Caffeicacid is selected here as the best lead against Serine protease.

Table 2. Docked result of selected hits from *Piper nigrum* against venom proteins

Targets	Ligands	BE	IC(uM)	H b	Bond type	BL	Residue
ACE	Beta cubebine	-6.61	14.34	4	OHN OHN OHO OHN	2.9 3.0 3.1 3.0	Ala77 Asn129 Ser134 Gly136
	Beta sitosterol	6.19	29.04	1	OHO	2.7	Lys 130
	Campesterol	-6.00	41.23	0			
	Cedrol	-5.91	46.73	1	OHO	3.0	Glu75
	Epoxy dihydro caryophyllene	-5.88	48.98	1	OHN	2.7	Gly136
CA	1-epi-cubenol	-5.70	66.76	1	OHO	2.54	Tyr51
	A-copaene	-5.14	171.10	0			
	Caryophyllene oxide	-5.13	174.00	0			
	Germacrene B	-5.59	80.32	0			
	Alpha gurjunene	-5.48	95.92	0			
CB	Oxalic acid	-5.93	205.23	1	OHN	2.5	Lys12
	T-muurolol	-5.80	56.44	1	OHO	2.4	Pro30
	Epicubenol	-5.56	84.61	2	OHN OHO	2.8 3.2	Arg36 Asn60
	Cubenol	-5.50	93.49	2	OHN OHO	2.9 3.2	Arg36 Asn60
	Cubebol	-5.35	120.40	2	OHO OHN	2.3 2.6	Arg36 Asn60
CBT	Betasitosterol	-6.18	81.82	0			
	Campesterol	-6.11	33.49	1	OHO	2.51	Asn5
	17-Beta estriol	-5.82	54.20	1	OHO	2.60	Asn5
	Cubebin	-5.38	113.56	1	OHO	3.16	Cys41
	Trichostachine	-5.31	127.76	2	OHN OHO	2.72 2.6	Arg36 Thr37
CYT	Stigmasterol	-6.77	10.98	0			
	Betasitosterol	-6.11	33.13	0			
	Campesterol	-6.11	33.16	0			
	Betacubebin	-6.09	34.38	0			
	Retrofractamidea	-6.00	41.47	0			
LAA	Betasitosterol	-9.90	.05573	1	OHO	2.7	Ser445
	Stigmasterol	-9.04	.23637	1	OHO	3.13	Arg90
	Beta cubebin	-8.77	.37209	2	OHN OHN	2.89 3.34	Lys343 Met108
	Retrofractamidea	-8.56	.53061	2	OHN OHN	2.52 2.96	Met108 Arg109
	Quercitrin	-8.16	1.05	8	OHO OHO OHO OHN OHO OHN OHN OHO	2.6 2.8 3.23 3.04 2.62 2.88 3.04 2.99	Ser445 Ser445 Ser445 Thr447 Gly444 Arg109 Lys343 Arg109
LN1	1- Terpinen-5-OL	-6.00	39.94	1	OHO	2.6	Pro64
	Alpha selinene	-5.79	56.67	0			
	Alphacubebene	-5.67	69.79	0			

	Piperitone	-5.82	54.33	0			
	Isochavinicacid	-5.81	55.01	2	OHO OHN	2.43 2.93	Arg70 Arg70
LN2	Betasitosterol	-7.09	6.37	1	OHO	2.8	Asp38
	Campesterol	-6.34	22.5	1	OHO	2.9	Gly34
	Betacubebine	-6.19	29.2	3	OHO OHN OHN	2.7 2.6 2.3	Val37 Val37 Lys69
	17beta estriol	-6.16	30.7	1	OHO	2.7	Asp27
	(5,10,15)-Cadinen-4-ol	-6.15	30.8	2	OHO OHN	3.1 2.9	Val37
LN3	1-Terpinen-4-ol	-5.84	52.23	2	OHO OHO	3.0 2.7	Cys62 Pro64
	Piperitone	-5.83	53.70	0			
	Alphacubebene	-5.80	56.45	0			
	Betaselinene	-5.76	60.26	0			
	1,8-P-Menthadien-5-ol	-5.68	68.85	1	OHO	2.5	Pro64
LN4	1-terpinen-5-ol	-6.00	39.94	1	OHO	2.6	Pro64
	Piperitone	-5.82	54.33	0			
	Isochavinicacid	-5.81	55.01	2	OHO OHN	2.43 2.93	Arg70 Arg70
	Alpha selinene	-5.79	56.67	0			
	Alpha cubebene	-5.67	69.79	0			
LN5	Betasitosterol	-7.09	6.37	1	OHO	2.8	Asp38
	Campesterol	-6.34	22.51	1	OHO	2.9	Gly34
	Betacubebine	-6.19	29.25	3	OHO OHN OHN	2.7 2.6 2.3	Val37 Val37 Lys69
	17beta estriol	-6.16	30.77	1	OHO	2.7	Asp27
	(5,1015)-Cadinen-4-ol	-6.15	30.83	2	OHO OHN	3.1 2.9	Val37 Val37
PLA2	Betasitosterol	-11.46	.00399	1	OHO	3.33	Phe21
	Campesterol	-10.51	.01968	0			
	Guineensine	-10.02	.04527	1	NHO	2.96	Cys44
	6-Trans-piperamide	-9.00	.28872	0			
	Betacubebine	-8.53	.56321	2	OHO OHN	2.76 3.02	Asn52 Tyr63
Proteolase	Betasitosterol	-6.93	8.29	2	OHO OHN	1.5 1.9	Leu385 Lys387
	Cubebin	-6.59	14.82	4	OHO OHN OHN OHN	3.0 2.7 2.9 3.0	Asn301 Lys387 Glu192 Arg455
	Campesterol	-6.57	15.39	2	OHO OHO	3.1 2.7	Gln190 Arg187
	Piperine	-6.35	26.06	1	OHN	2.6	Glu440
	Isochavicine	-6.25	26.41	1	OHN	2.6	Glu440
Serine protease	Pcoaricacid	-7.36	4.02	2	OHO OHO	3.12 2.64	Thr42 His38
	Isochavinicacid	-6.92	8.42	1	OHN	2.61	Lys34
	Caffeicacid	-6.58	15.09	6	OHO	2.99	Thr35

					OHO	3.27	Thr39
					OHO	2.73	Thr39
					OHO	2.53	Asn33
					OHN	2.66	Lys46
					OHN	2.68	Lys43
	Cinnamicacid	-6.43	19.30	2	OHN	2.52	Lys46
					OHN	2.73	Lys43
	Campesterol	-6.17	29.79	2	OHO	2.91	Leu40
					OHN	2.63	Leu40

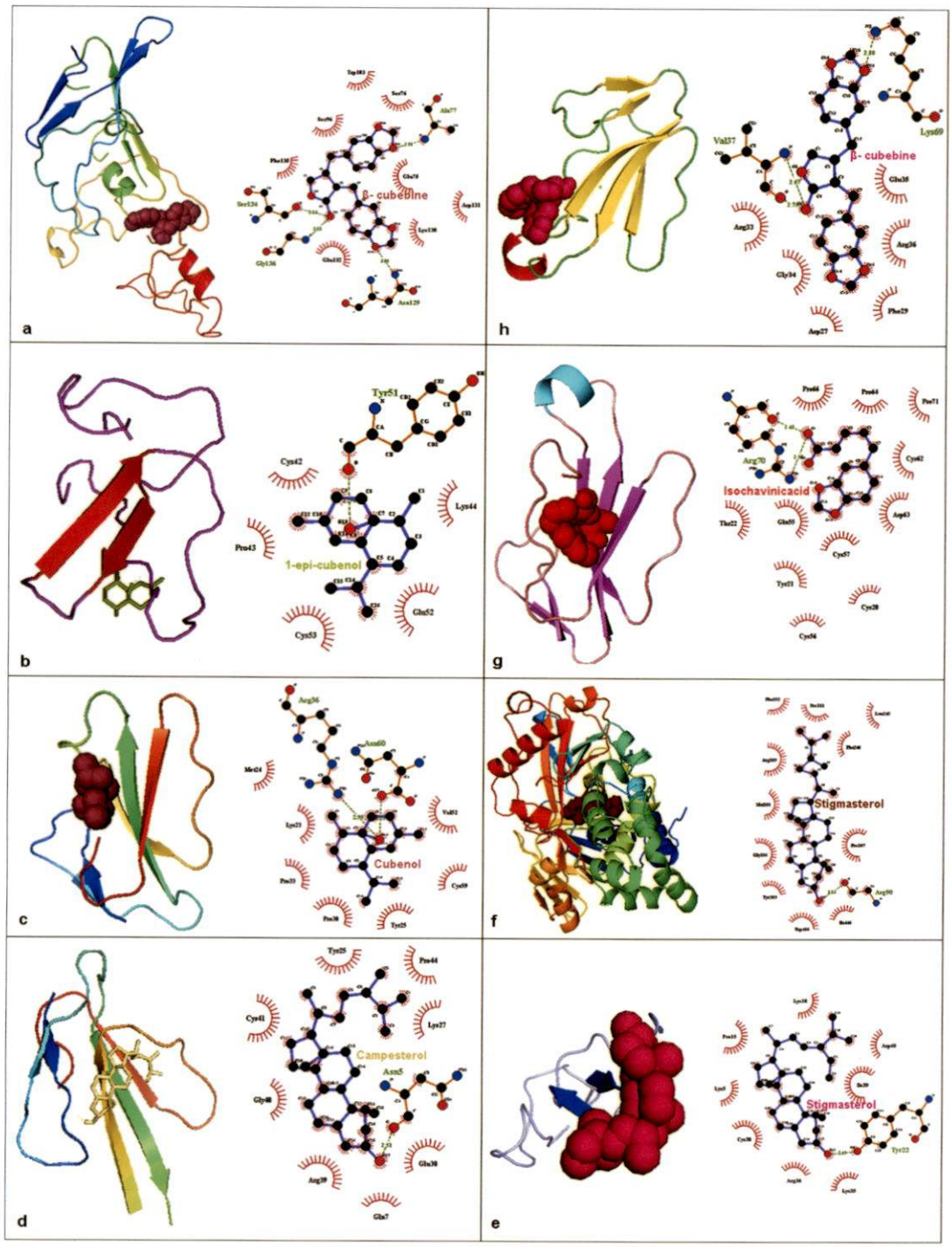

Figure 4. Docked poses of target and leads from *Piper nigrum* in pymol viewer and ligplot.

a. ACE and Beta cubebine, b. CA and 1-epi Cubenol, c. CB and Cubenol, d. CBT and Campesterol, e. CYT and Stigmasterol, f. LAAO and Stigmasterol, g. LN1 and Isochavinic acid, h. LN2 and Beta cubebine.

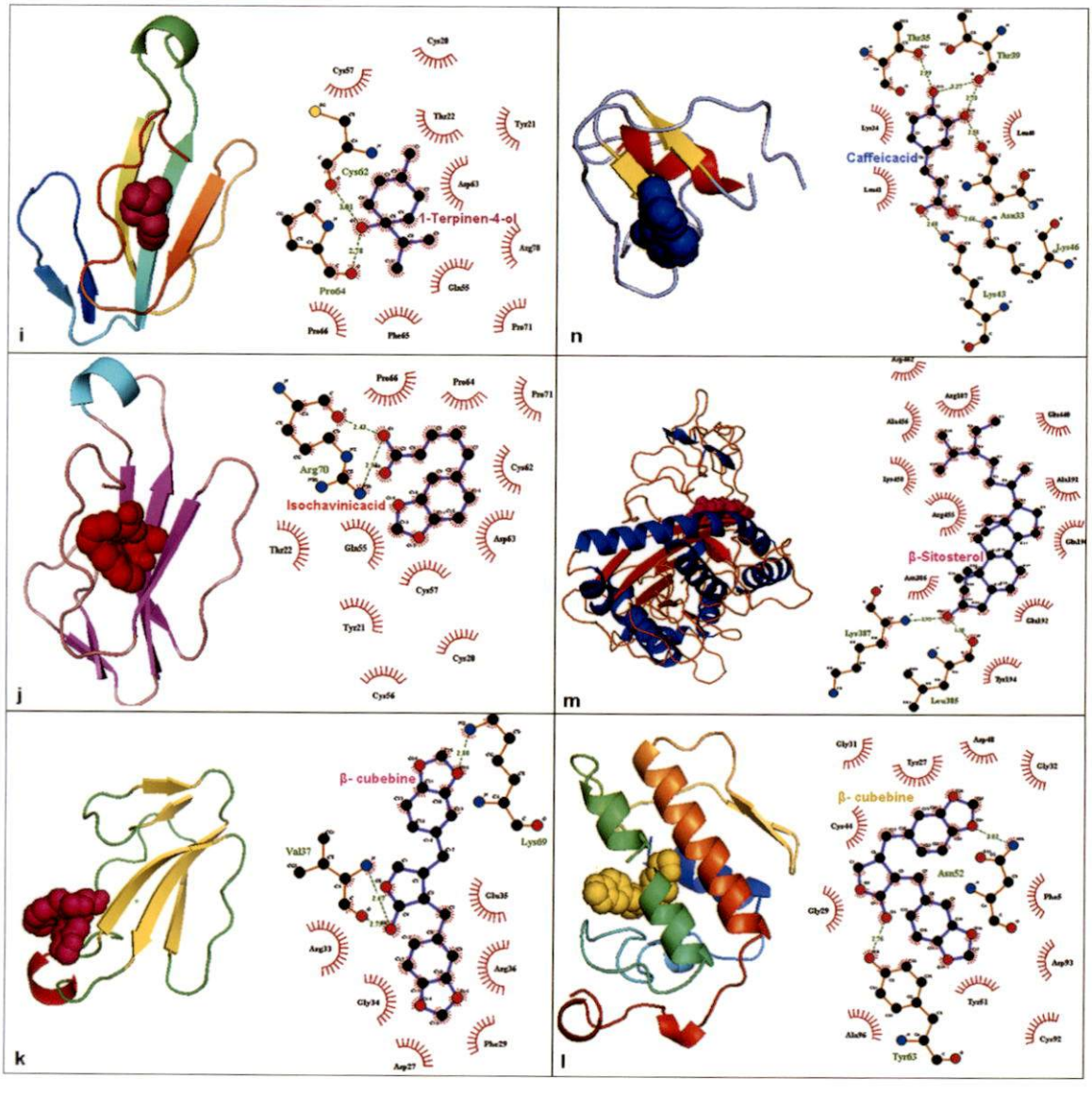

Figure 4. Continued.

i. LN3 and 1- Terpine-4-ol, j. LN4 and Isochavinic acid, k. LN5 and Beta cubebine, l. PLA2 and Beta cubebine, m. PRO and beta sitosterol, n. SER and Caffeic acid.

4.3 DOCKED RESULTS OF *CINNAMOMUM ZEYLANICUM* BLUME.

Cinnamon is a reputed spice derived from an evergreen small tree *Cinnamomum zeylanium* belongs to the family Lauraceae. Through literature review 128 constituent compounds so far reported were selected here as ligands for screening the toxic venom proteins. Lead obtained from each target protein (table-3) is discussed here in detail.

About 8 molecules showed free energy of binding <= -5Kcal/mol against Acetyl cholinesterase. Among them, compounds with most least free energy of binding such as Campesterol, Betaselinene, Gammacadinene, Alphahulene and Trans calamemene were screened as top hits. None among the hit molecules formed H-bond interaction with the target. Campesterol is the top hit molecule here with least free energy of binding -6 Kcal/mol and posses lowest inhibition constant (41.23 micromolar). Campesterol possessed better Hydrodrophobic interaction than other hits. Thereby Campesterol is selected here as the best lead among the hits.

Against Cobramin A , Only 5 molecules showed free energy of binding less than -5 kcal/mol. They selected as hits . They are 7-epi-a-selinene, A-cadinol, Alpha copaene, Caryophyllene oxide and Beta elemene. Among these Compounds only Alphacadinol showed a hydrogen bond interaction at the residue Arg36 and selected Alphacadinol as the best lead. It also promises with drug likeness properties according with Lipinski's rule of five.

Docking of Cobramine B determined 10 phytomolecules with the free energy of binding <= -5Kcal/mol. Among them α-Muurolol, (epi-α) Cadinol, Cyanidin, Alpha ylangene and Gammacadinene are the compounds with most least free energy of binding and selected them as top hits. Alpha cadinol and Alpha muurolol formed single hydrogen bonds each at the same residue Pro30. Other hits didn't formed any H-bond interacton. Considering the least free energy of binding and inhibition constant level Alpha cadinol is selected as the best lead. The Lys 23 which is the critical residue for the target protein is also involved in

the hydrophobic interaction with the ligand and showed no violation in the Lipinski's rule of five.

About 18 compounds against Cobrotoxin were shown binding affinity with free energy level below -5 Kcal/mol. Of them 5 molecules such as Curcuminoid, α -Muurolene, Phytosterol, Campesterol and Proanthocyanidin-B5 were screened as top hits in accordance with the order of lowest ΔG_{bind}. Canpesterol and Proanthocyanidin-B5 showed single H-bond each at the residue Asn5 followed by Curcuminoid formed 3 H-bonds of OHN bond type. Other ligands didn't establish any H-bond interaction with the target. Considering the least free energy of binding, more number of H-bonds the ligand Curcuminoid is selected here as the best lead. It also fully obeyed Lipinski's rule of five.

In the case of Cytotoxin, about 12 compounds were showing binding affinity with ΔG_{bind} <=-5Kcal/mol. Among them 5 molecules were considered for further analysis. They are Curcuminoid, Campesterol, Gammacadinene, Betaselinene and P-coaric acid No hits formed H-bond interaction with the protein except Curcuminoid and P- Coumaric acid. P-coumaric acid with the highest ΔG_{bind} hit formed an H-bond with the residue Cys38. Curcuminoid formed 2 H-bonds of OHN bond type with the residues Lys18, Val 41. Of which Lys 18 is the critical residue for the protein Cytotoxin. It also obeyed the Lipinski's rule of five. Therefore Curcuminoid is selected here as the best lead against Cytotoxin.

Out of 92 active molecules (ΔG_{bind} <= -5Kcal/mol) against L-aminoacid oxidase 5 hits such as Curcuminoid, Campesterol, Proanthocyanidin-B1, Proanthocyanidin and Proanthocyanidin-B2 were screened based on the order of least free energy of binding. Except Campesterol all the ligands were well interacted and formed 3 or more H-bonds. Curcuminoid has 3 hydrogen bond at the residues Met62, Lys343 and Arg 109. ProanthocyanidinB1and Proanthocyanidin formed 6 H-bonds each and ProanthocyanidinB2 formed 5 H-bonds but molecular weight of these compounds are exceeding the 500Kdalton limit. Therefore Curcuminoid is selected as the best lead considering its least free

energy of binding, lowest inhibition constant and considerable number of H-bonds (3 nos.) of OHN bond type.

In the case of Long Neurotoxin 1, about 30 compounds were showing binding affinity with ΔG_{bind} <= -5Kcal/mol. Among them 5 molecules were screened as top hits they are, Piperitone, Alphaterpineol, Gamma terpinene, Beta selinene and Limonene based on the order of least free energy of binding. Except Alpha Terpineol all other ligands didn't formed H-bond interactions. Alpha Terpineol with considerable least free energy of binding level and inhibition constnat formed one hydrogen bond interaction at the Pro64. Therefore it is considered as the best lead molecule against Long neutotoxin 1.

Five hit molecules from 30 active molecules against Long neurotoxin 2 were screened based on their level of free energy of binding. They are Campesterol, Curcuminoid, Alphaylangene, Alphaphellandrene and Caryophylleneoxide. Campesterol and Curcuminoid formed H-bond interaction with the target protein. Campesterol formed one hydrogen bond with the residue Gly34. While, Curcuminoid formed 2 hydrogen bonds with the active site residues Arg33, Val37. The other three ligands didn't form any H-bond interaction. Therefore Curcuminoid is selected as the best lead after considering its least free energy of binding (-6.20 Kcal/mol), lower inhibition constant (28.40) and higher number of H-bond interacton (2 nos.).

About 30 molecules showed free energy of binding <= -5 Kcal/mol against Long Nuerotoxin 3. Among them 5 molecules were considered as the top ranked hit molecules. They are Alphaphellandrene, Betaselinene, Gammaterpineol, Cinaldehyde and Piperitone. None of them showed hydrogen bond interaction with the protein. So Piperitone is taken as the lead with least free energy of binding and lowest inhibition constant. Its drug likeness properties are also in acceptable range.

Five hit compounds from 30 active molecules against Long neurotoxin 4 were screened based on their level of free binding energy. The selected hits are

Piperitone, Alphaterpineol, Gammaterpinene, Betaselinene and Limonene. Among the hits Alpha Terpineol alone formed one hydrogen bond interaction with the Pro64 residue, which is an active site residue for the protein. So Alpha terpineol is selected as the best lead after considering its acceptable free energy of binding level, lower inhibition constant and H-bond interaction with the active site residue.

Thirteen molecules were shown binding activity against Long neurotoxin-5 with free energy of binding <= -5 Kcal/mol. Out of them Campesterol, Curcuminoid, Alphaylangene, Alphaphellandrene and Caffeicacid were selected as top hits based on the least free energy of binding. Only campesterol and curcuminoid formed hydrogen bond interactions with the target protein. Campesterol formed one hydrogen bond with the residue Gly34 while, Curcuminoid formed 2 hydrogen bond with residues Arg33 and Val37. Both these residues are in the active site of the target. They also involved in the hydrophobic interaction with the protein. So curcuminoid is the best lead against the target Long neurotoxin-5.

Five hit molecules from 96 active molecules aginst Phospholipase A2 were screened based on their level of binding energy. Five hits such as Campesterol, Curcuminoid, (epi-α) Bisabolol, Cadalene and Betaselinene were selected based on their order of least free energy of binding leve. None of the ligand compounds except Curcuminoid formed hydrogen bonds with the residues of the target. Curcuminoid formed single hydrogen bond at the residues His 47. HIS47 which is an active residue for phospholipase A2. So Curcuminoid is selected as the lead.

About 8 molecules were shown binding activity against Proteolase , Out of them 5 molecules were selected as top ranked hits. They are Campesterol, Curcuminoid, Epicatechin, Phytosterol and Cyanidin. Campesterol and Curcuminoid formed two H-bonds each followed by Cyanidin and Phytosterol formed single H-bonds each while 3 H-bonds were formed between Epicatechin and the target. One of the H-bonds between the Curcuminoid is with the critical

residue Asp47. Though Campesterol is the hit compound with least free energy of binding, the second most top hit Curcuminoid which established one of the H-bond interaction at the critical residue with acceptable free energy of biniding (-6.16 Kcal/mol) & inhibition constant (30.38) were selected here as the best lead. The lead also fully agreed with the Lipinski's rule of five.

In the case of Serine protease, about 9 compounds were showing binding affinity with ΔG_{bind} <-5Kcal/mol. Among them 5 molecules with most least free energy of binding were screened as hits. They are Curcuminoid, P-coumaricacid, Caffeicacid, Campesterol and Alphaylangene. Alphaylangene has no hydrogen bond while Caffeic acid established two hydrogen bond at the residues Thr35 and Thr39. Followed by Campesterol fomed 2 hydrogen bond at the same residue Leu40. Curcuminoid showed 3 hydrogen bonds at Lys46, Lys43 and Thr39. P-Coumaric acid exhibited 4 hydrogen bond at Lys46, Lys43, Thr35 and Thr39. Among them Curcuminoid is the hit with least free energy of binging, lowest inhibition constant and established 3 H-bonds of OHN and OHO bond type, therefore Curcuminoid is selected here as the best lead.

Table 3. Docked result of selected hits from *Cinnamomum zeylanicum* against venom proteins.

Target proteins	Ligands	BE	IC(uM)	H b	Bond type	BL	Residue
ACE	Campesterol	-6.00	41.23	0			
	Betaselinene	-5.78	57.49	0			
	Gammacadinene	-5.43	104.51	0			
	Alphahulene	-5.40	109.80	0			
	Trans calamemene	-5.35	118.79	0			
COA	7-epi-a-selinene	-5.65	71.95	0			
	Alpha cadinol	-5.51	91.42	1	OHO	2.69	Arg36
	Alpha copaene	-5.14	171.10	0			
	Caryophyllene oxide	-5.13	174.00	0			
	Beta elemene	-5.02	207.45	0			
COB	α-Muurolol	-5.80	56.44	1	OHO	2.4	Pro30
	(epi-α) Cadinol	-5.79	56.74	1	OHO	2.5	Pro30
	Cyanidin	-5.47	98.40	0			
	Alphaylangene	-5.31	128.00	0			
	Gammacadinene	-5.25	142.02	0			
CBT	Curcuminoid	-8.39	712.82	3	OHN OHN OHN	2.73 2.98 3.32	Lys27 Lys47 Gly49
	α -Muurolene	-7.06	6.71	0			
	Phytosterol	-6.83	9.83	0			
	Campesterol	-6.11	33.49	1	OHO	2.51	Asn5
	Proanthocyanidin-B5	-5.77	58.74	1	OHO	2.56	Asn5
CYT	Curcuminoid	-7.35	4.07	2	OHN OHN	3.01 3.12	Lys18 Val41
	Campesterol	-6.11	33.16	0			
	Gammacadinene	-5.34	120.94	0			
	Betaselinene	-5.27	137.57	0			
	P-Coaric acid	-5.14	171.08	1	OHO	2.83	Cys38
LAA	Curcuminoid	-10.32	.02745	3	OHN OHN OHN	3.12 2.79 3.06	Met62 Lys343 Arg109
	Campesterol	-10.07	.04189	0			
	Proanthocyanidin-B1	-8.09	1.18	6	OHO OHO OHN OHO OHO OHN	2.95 3.26 2.91 2.65 2.76 2.90	Arg109 Thr447 Thr447 Gly106 Tyr389 Lys343
	Proanthocyanidin	-8.07	1.21	6	OHO OHN OHN OHN OHO OHN	2.95 3.13 2.90 3.07 2.66 2.92	Ser445 Thr447 His114 Arg109 Arg109 Arg109
	Proanthocyanidin-B2	-7.96	1.47	5	OHO OHN OHO OHO OHN	2.60 3.23 2 1.53 2.70	Arg109 Thr447 Tyr389 Gly106 Lys343

LN1	Piperitone	-5.82	54.33	0			
	Alphaterpineol	-5.76	59.46	1	OHO	2.43	Pro64
	Gamma terpinene	-5.69	67.52	0			
	Beta selinene	-5.62	76.40	0			
	Limonene	-5.49	94.43	0			
LN2	Campesterol	-6.34	22.51	1	OHO	2.94	Gly34
	Curcuminoid	-6.20	28.40	2	OHN OHN	2.24 3.10	Val137 Arg33
	Alphaylangene	-5.56	83.79	0			
	Alphaphellandrene	-5.46	99.05	0			
	Caryophyllene oxide	-5.42	106.16	0			
LN3	Piperitone	-5.83	53.70	0			
	Gammaterpineol	-5.79	56.96	0			
	Betaselinene	-5.76	60.26	0			
	Alphaphellandrene	-5.63	74.21	0			
	Cinaldehyde	-5.49	94.40	0			
LN4	Piperitone	-5.82	54.33	0			
	Alphaterpineol	-5.76	59.46	1	OHO	2.43	Pro64
	Gammaterpinene	-5.69	67.52	0			
	Betaselinene	-5.62	76.40	0			
	Limonene	-5.49	94.43	0			
LN5	Campesterol	-6.34	22.51	0			
	Curcuminoid	-6.20	28.40	2	OHN OHN	2.24 3.10	Val137 Arg33
	Alpha ylangene	-5.56	83.79	0			
	Alpha phellandrene	-5.46	99.05				
	Caffeicacid	-5.38	113.39	1	OHN	3.10	Arg33
PLA2	Campesterol	-10.51	.01968	0			
	Curcuminoid	-9.21	.17739	1	OHN	2.6	His47
	(epi-α) Bisabolol	-8.27	.868	0			
	Cadalene	-7.89	1.65	0			
	Betaselinene	-7.60	2.70	0			
PRO	Campesterol	-6.57	15.39	2	OHO OHO	3.13 2.73	Gln190 Arg187
	Curcuminoid	-6.16	30.38	2	OHN OHN	2.84 3.03	Lys458 Asp477
	Epicatechin	-5.88	49.32	3	OHO OHO OHO	2.63 2.61 2.93	Cys474 Cys474 Asp462
	Phytosterol	-5.78	57.71	1	OHO	2.43	Asp462
	Cyanidin	-5.36	117.56	1	OHN	2.33	Lys458
SER	Curcuminoid	-7.39	3.84	3	OHN OHN OHO	3.47 2.36 2.93	Lys46 Lys43 Thr39
	P-coumaricacid	-7.36	4.02	4	OHN OHN OHN OHO	2.53 2.62 2.96 2.79	Lys46 Lys43 Thr35 Thr39
	Caffeicacid	-6.58	15.09	2	OHO OHO	2.99 3.27	Thr35 Thr39
	Campesterol	-6.17	29.79	2	OHO OHN	2.91 2.63	Leu40 Leu40
	Alpha ylangene	-5.61	77.18	0			

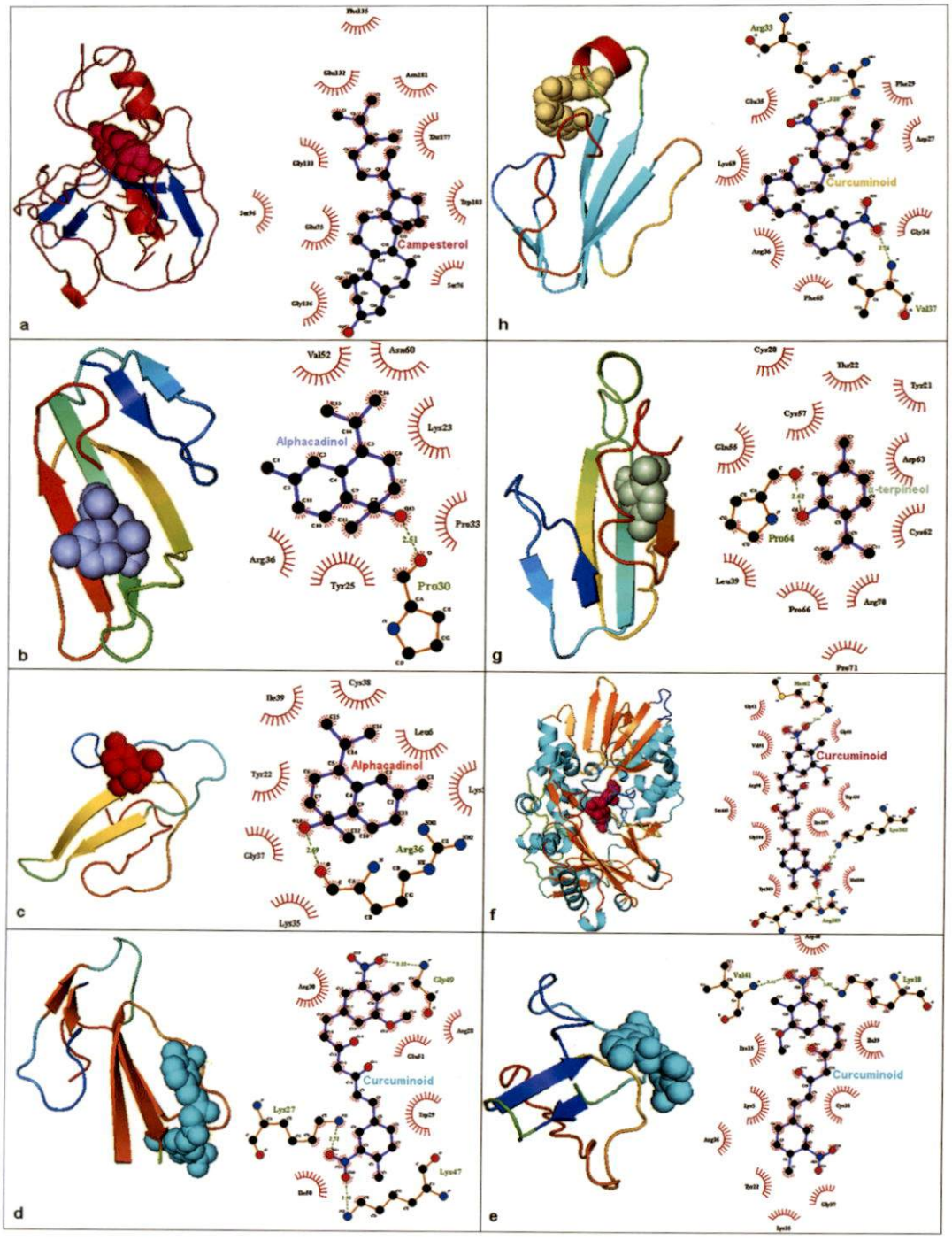

Figure 3. Docked poses of target and leads from *Cinnamomum zeylanicum* in pymol viewer and ligplot.

a. ACE and Campesterol, b. CA and Alpha Cadinol, c. CB and Alpha Cadinol, d. CBT and Curcuminoid, e. CYT and Curcuminoid, g. LN1 and Alpha Terpineol, h. LN2 and Curcuminoid.

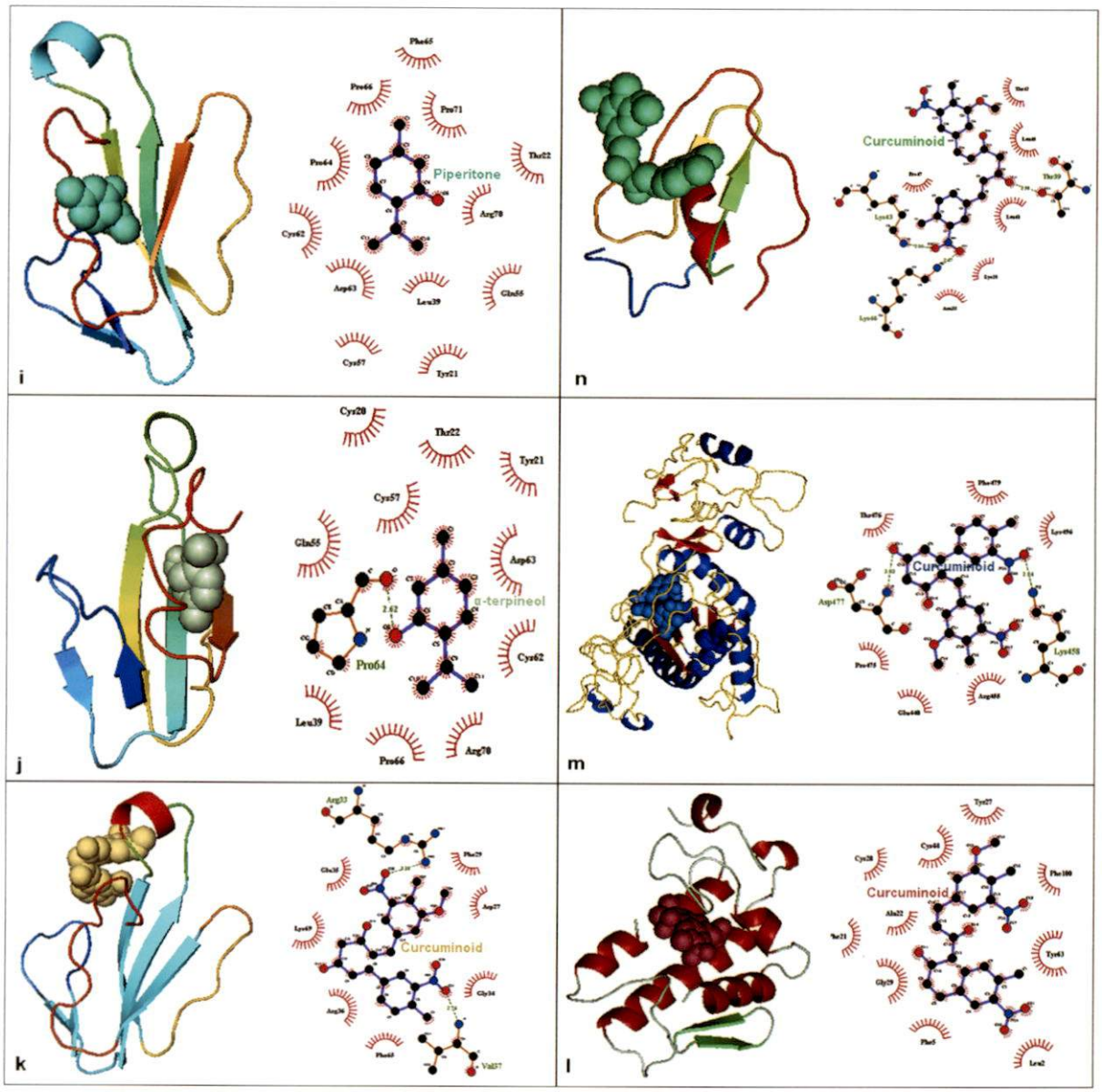

Figure 3. Continued.

i. LN3 and Piperitone, j. LN4 and alpha Terpineol, k. LN5 and Curcuminoid, l. PLA2 and Curcuminoid, m. Proteolase and Curcuminoid, n. Serine Protease and Curcuminoid.

4.4 DOCKED RESULT OF *CAPSICUM FRUTESCENS*

Capsicum frutescens is a perennial small shrub belongs to the family Solanaceae, native to tropical America. Detailed examination of the so far published constituent phytomolecules from the plant about 120 phytochemicals was selected for the present study. Lead obtained from each target protein (table-4) is discussed here in detail.

Only four molecules showed free energy of binding <= -5 Kcal/mol against Acetyl cholinesterase. They are Solanidine, Solasodine, Campesterol and Betacaryophyllene. Solanidine formed one hydrogen bond at the residue Phe73. While, Solasodine formed 2 hydogen bonds with Glu75. Other molecules didn't estabhlish any interaction with the protein. Among these compounds Solasodine is selected as the lead with 2 hydrogen bonds of NHO bond type and acceptable range of free energy of binding & lower level of inhibition constant. The lead also showed hydrophobic interaction with the critical residue Ser76.

Only 2 molecules showed free energy of binding less than -5 Kcal/mol against Cobramine B. They are Caryophyllene (-5.98Kcal/mol), Malonic acid (-5.49 Kcal/mol). Caryophyllene has no hydrogen bond with protein, followed by Malonic acid formed 3 hydrgen bond of OHN bond type at the residues Cys38 and Lys18. Therefore Malonic acid is selected as the best lead which showed no violation in the Lipinski's rule of five.

Only 3 molecules showed free energy of binding <= -5 Kcal/mol against Cobrotoxin. They are Solanidine (-7.63 Kcal/mol), Solasodine (-6.20 Kcal/mol), Campesterol (-6.11 Kcal/mol). Solanidine showed one hydrogen bond with the active site residue Asp31, followed by Solasodine formed 2 hydrogen bond at Asp 31 and His32. Campesterol established single hydrogen bond at Asn5. Solanidine is the hit with least free energy of binding and lowest inhibition constant. It also hydrophobically interacted with the critical residue Arg33. Therefore Solanidine is selected as the best lead against Cobrotoxin.

Against Cytotoxin 5 molecules showed free energy of binding less than -5kcal/mol. They are Campesterol, Caryophellene alcohol, Solanidine, Kaempferol and P-coumaric acid. Campesterol and Caryophellene formed 2 H-bonds each with Leu6 and Arg36. Kaempferol formed 4 H-bonds and P-coumaric acid formed single H-bond but higher level of ΔG_{bind}. Campesterol shown least free energy of binding and lowest inhibition constant among the hits. Considering its acceptable number of H-bond interaction with active site residues and hydrophobic interaction with the critical residue Lys18 Campesteorl is selected here as the best lead against Cytotoxin. The lead is also agreed with the Lipinski's rule of five.

Against L amino acid oxidase 53 compounds showed free energy of binding <= -5 Kcal/mol. Among which five molecules with least free energy of binding were selected as top hits. They are Campesterol, Solanidine, Solasodine, Eriodictin and Apiin. Campestrol formed one hydrogen bond at Arg90 whch is the critical residue here. Solanidine and Solasodine also formed single hydrogen bonds each. The other two molecules formed 2 and 3 H-bonds respectively but with higher free energy of binding. Considering the least free energy of binding, Hydrogen bond interaction with the critical residue and the lowest molecular weight among the hits Campesterol is selected as the best lead against Lamino acid oxidase.

Longnuerotoxin1 resulted 14 molecule with free energy of binding less than -5 Kcal/mol. 5 molecules were selected as hits for further analysis. They are 1-Terpinen-4-ol, Octopamine, Betaionone, Limonene and Isothujone. Hydrogen bond interaction is absent in 1-Terpinen-4-ol, Betaionone, Limonene and Isothujone. Octopamine formed 2 hydrogen bonds with Pro71 and one with Gln55 . Therefore Octopamine is selected as the best lead though 1-Terpinen-4-ol showed least free energy of binding . It also obeyed lipinskis solubility and permeability rules. Against Longnuerotoxin3 , About 15 molecules showed free energy of binding less than -5 Kcal/mol . Among them 5 molecules with least free energy of binding were selected as hits. They are 1-Terpinen-4-ol, Octopamine,

Betaionone, betaphellandrene and Vanillylamine. 1-Terpinen-4-ol formed two hydrogen bonds at Cys62 and Pro64, followed by Octopamine fomed 3 hydrogen bonds at Pro71and Cys62, Hydrogen bond interaction is absent in Betaphellandrene and Beta Ionone. While VanilylAmine established 2 hydrogen bond with the residues Pro71 and Cys62. Considering the acceptable least free energy of binding & inhibition constant, higher number of hydrogen bonds and agreeable dug likeness properties of the ligand Octopamine is selected as the best lead against LN3.

Only 4 molecules showed free energy of binding <= -5 Kcal/mol against Long nuerotoxin4. They are Beta ionone (-5.46), Octopamine (-5.14), Vanilyamine (-5.06) and Pulegone (-5.03). H-bond interaction is absent in Beta ionone and Vanillyl Amine. Pulegone formed single hydrogen bond at the residue Thr22 followed by Octopamine established 3 H-bonds with Pro66, Pro66 and Gln55. Considering the lower molecular weight, more number of H-bond interaction and second most least free energy of binding Octopamine is selected as the best lead against Longneurotoxin4.

In the case of Long Neurotoxin 5, ten compounds were showing binding affinity with ΔG_{bind} <=-5Kcal/mol. Out of them Campesterol (-6.34 Kcal/mol), Betaphellandrene (-5.46 Kcal/mol), Caffeic acid(-5.38 Kcal/mol), Solanidine (-5.25 Kcal/mol) and Solasodine (-5.13Kcal/mol) were considered as top hits. The least and highest free energy of binding compounds Campesterol and Solasodine respectively established single H-bonds each with the same residue Gly34 followed by Solanidine formed 2 H-bonds with Val37. Caffeicacid formed 3 H-bonds with Asp27 and with the critical residue Arg33. Considering the lowest molecular weight more number of H-bonds and the interaction with the critical residue Caffeicacid is selected here as the best lead against LN5.

In the case of PLA2 about 100 compounds were showing binding affinity with ΔG_{bind} <= -5Kcal/mol. Among them compounds with least free energy of binding such as Phylloquinone, Solanidine, Norcapsaicin, Novivamide and Solasodine were selected as top hits. H-bond interaction is absent in

Phylloquinone. Norcapsaicin and Solasodine formed single H-bonds each followed by Novivamide and Solanidine formed 2 hydrogen bonds each. Since the compound with least free energy of binding, Phylloquinone not established any bond interaction the second most least free energy of binding compound Solanidine with two H-bonds, lower molecular weight and lower inhibition constant were selected here as the best lead against PLA2.

In the case of Proteolase 15 compounds were showing binding affinity with ΔG_{bind} <= -5Kcal/mol. Among them compounds with least free energy of binding such as Campesterol, Quercitin, Solanidine, Kaempfero and Citrullin were selected as top hits for further analysis. Solanidine and Campesterol possessed two hydrogen bonds each with the target. Three hydrogen bonds are formed by Kaempferol while 5 H-bonds are established by Citrullin. Quercetin is the second most free energy of binding compound formed the highest number of six hydrogen bond at the residues Lys387, Glu439, Glu440, and Arg455. Therefore Quercetin is selected as the best lead as it showed somewhat equal level of ΔG_{bind} and inhibition constant to the top hit with higher number of H-bonds. The lead also obeys Lipinski's rule of five.

Against Serine protease 10 compounds were showing binding affinity <= -5Kcal/mol. Among these Ferulic acid (-7.48Kcal/mol), Caffeic acid(-6.58Kcal/mol), Malonic acid (-6.22 Kcal/mol), Solanidine (-5.73Kcal/mol) and Solasodine (-5.35Kcal/mol) with least free energy of binding were screened as top hits. Ferulic acid formed 2 hydrogen bonds with the residues Lys46 & Lys43 and 2 bonds with Thr39. Followed by Malonic acid and Solasodine formed 3 hydrogen bonds each while Solanidine has no hydrogen bond interaction. Caffeic acid established 6 hydrogen bonds. Considering the least free energy of binding and lowerst inhibition constant with considerable number of H-bond interactions with active site residues, Malonic acid is selected as the best lead against Serine protease. The lead obeys the Lipinski's rule of five.

Table 4. Docked result selected hits from *Capsicum frutescens* against venom proteins

Targets	Ligands	BE	IC(uM)	H b	Bond type	BL	Residue
Acetyl cholineesterase	Solanidine	-6.22	27.81	1	OHO	2.71	Phe73
	Solasodine	-6.14	31.73	2	NHO NHO	2.65 2.94	Glu75 Glu75
	Campesterol	-5.98	41.23	0			
	Betacaryophyllene	-5.69	67.32	0			
Cobramine B	Caryophy-llene	-5.98	41.23	0			
	Malonic acid	-5.49	94.35	3	OHN OHO OHN	2.81 2.78 2.58	Cys38 Cys38 Lys18
Cobrotoxin	Solanidine	-7.63	2.57	1	OHO	2.93	Asp31
	Solasodine	-6.20	28.36	2	NHO OHN	3.23 2.78	Asp31 His32
	Campesterol	-6.11	33.49	1	OHO	2.51	Asn5
Cytotoxin	Campesterol	-6.11	33.16	2	OHO OHN	3.13 2.79	Leu6 Arg36
	Caryophellene alcohol	-5.66	71.53	2	OHN OHO	3.06 2.64	Leu6 Arg36
	Solanidine	-5.51	90.90	0			
	Kaempferol	-5.38	114.63	4	OHN OHN OHO OHN	3.03 3.18 2.60 3.05	Leu6 Leu6 Arg36 Lys35
	P-coumaric acid	-5.14	171.08	1	OHO	2.83	Cys38
Laminoacid oxidase	Campesterol	-10.07	.4189	1	OHO	3.13	Arg90
	Solanidine	-9.16	.19294	1	OHO	2.65	Ser445
	Solasodine	-8.97	.26512	1	OHO	3.12	Gly444
	Eriodictin	-8.78	.36516	2	OHN OHO	2.16 2.52	Lys343 Gly444
	Apiin	-7.68	2.35	3	OHN OHN OHN	2.73 2.97 3.03	Lys343 Arg109 His114
LN1	1-Terpinen-4-ol	-6.00	39.94	0			
	Octopamine	-5.76	59.68	3	NHO OHO OHN	2.50 3.10 2.94	Pro71 Pro71 Gln55
	Betaionone	-5.69	67.85	0			
	Limonene	-5.49	94.43	0			
	Isothujone	-5.32	125.95	0			
LN3	1-Terpinen-4-ol	-5.84	52.23	2	OHO OHO	3.01 2.75	Cys62 Pro64
	Octopamine	-5.78	57.53	3	OHO NHO OHO	2 2 1.6	Pro74 Gln55 Pro64
	Betaionone	-5.75	60.83	0			
	betaphellandrene	-5.63	74.21	0			
	Vanillylamine	-5.37	116.62	2	NHO OHO	2.43 2.73	Pro71 Cys62
LN4	Beta ionone	-5.46	98.83	0			
	Octopamine	-5.14	170.68	3	OHO OHO OHO	2.44 2.35 2.6	Pro66 Pro66 Gln55

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	Vanillylamine	-5.06	195.50	0			
	Pulegone	-5.03	205.00	1	OHO	2.45	Thr22
LN5	Campesterol	-6.34	22.51	1	OHO	2.94	Gly34
	Betaphellandrene	-5.46	99.05	0			
	-Caffeicacid	-5.38	113.39	3	OHO OHO OHO	2.84 2.62 2.62	Arg33 Asp27 Asp27
	Solanidine	-5.25	141.89	2	NHO OHO	2.90 2.95	Val37 Val37
	Solasodine	-5.13	173.45	1	OHO	2.72	Gly34
PLA2	Phylloquinone	-8.91	.29219	0			
	Solanidine	-8.72	.40654	2	OHO OHN	3.20 3.04	Asn52 Asn52
	Norcapsaicin	-8.43	.65786	1	OHO	2.05	Trp18
	Novivamide	-8.24	.91274	2	OHO OHN	3.01 1.06	Trp18 Gly29
	Solasodine	-7.21	5.23 uM	1	OHO	2	Asn52
Proteolase	Campesterol	-6.57	15.39	2	OHO OHO	3.13 2.73	Gln190 Arg187
	Quercitin	-6.47	18.05	6	OHN OHO OHO OHN OHO OHO	3 2.20 2.52 2.38 2.62 2.38	Lys387 Glu439 Glu439 Glu440 Glu440 Arg455
	Solanidine	-6.00	40.91	2	OHO OHO	2.71 3.32	Ala456 Asp462
	Kaempferol	-5.78	58.18	3	OHO OHN OHN	2.65 3.10 2.67	Glu439 Glu440 Cys474
	Citrullin	-5.57	82.63	5	NHO NHO OHO NHO NHO	2.93 2.83 2.77 2.60 2.53	Pro475 Asp477 Asp462 Asp462 Glu465
Serine protease	Ferulicacid	-7.48	3.28	4	OHN OHN OHO OHN	2.49 2.62 2.56 3.07	Lys46 Lys43 Thr39 Thr39
	Caffeicacid	-6.58	15.09	6	OHO OHO OHO OHO OHN OHN	2.99 3.27 2.73 2.53 2.66 2.68	Thr35 Thr39 Thr39 Asn33 Lys46 Lys43
	Malonicacid	-6.22	27.51	3	OHN OHN OHN	2.58 2.59 2.57	Lys46 Lys46 Lys43
	Solanidine	-5.73	63.01	0			
	Solasodine	-5.35	120.14	3	OHO OHN	2.57 2.76	Lys46 Lys46

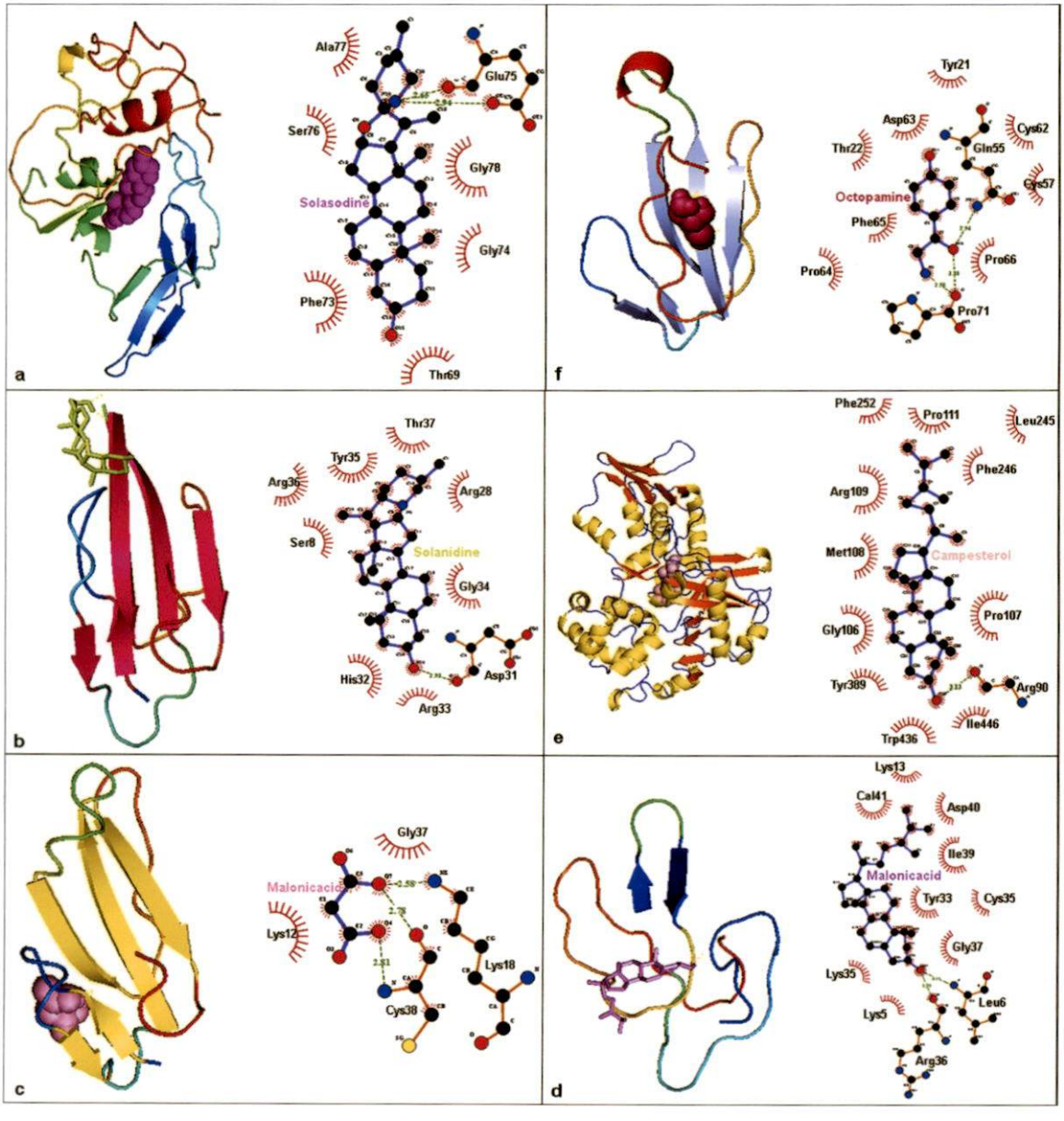

Figure 2. Docked poses of target and leads from *Capsicum frutescens* in pymol viewer and ligplot.

a. ACE and Solasodine, b. CBT and Solanidine, c. CB and Malonic acid, d. CYT and Campesterol, e. LAAO and Campesterol, f. LN1 and Octopamine,

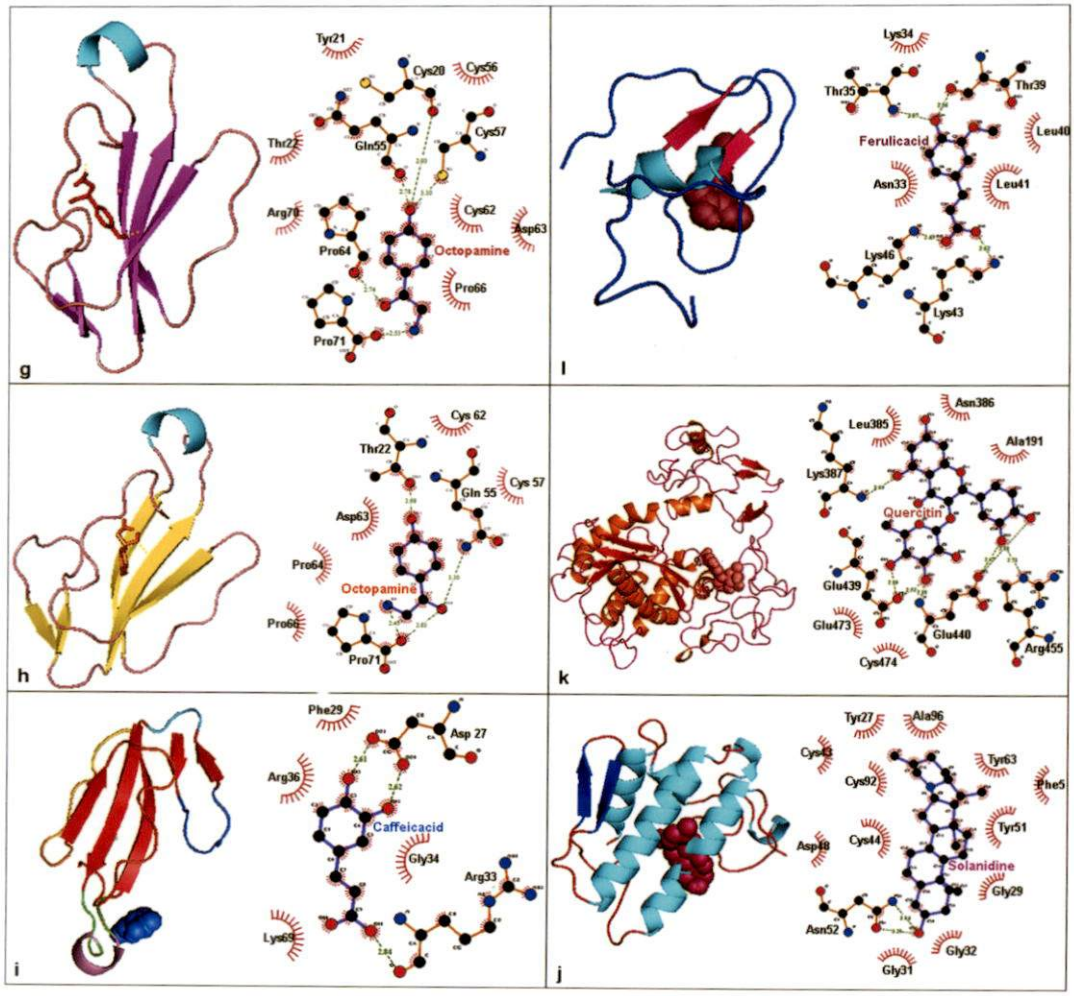

Figure 2. Continued.

h. LN4 and Octopamine, i. LN5 and Caffeic acid, j. PLA2 and Solanidine, k. PRO and Quercetin, l. SER and Ferulic acid.

4.5 CONSENSUS SCORING AND COMPARATIVE ANALYSIS OF PHYTOCHEMICALS USING DST METHOD

In accordance with DST, consensus scoring combines scores obtained from different docking tools based on distinct scoring functions to reduce errors in single scores and improve the probability of identifying true leads. The present study compared the top hits resulted in the scoring functions of PatchDock and Hex in addition to the AutoDock. Top ranked hits among the total 538 phytochemicals collectively present in the 4 spices, against each protein were subjected here for the analysis. The result revealed that the leads obtained in AutoDock such as curcuminoid, Betasitosterol, Isochavinic acid and 1-alpha epicubenol were the best leads. However certain hit molecules that are not included in the lead list of AutoDock result *viz.* 4- Alpha methyl zymosterol, Caryophyllene, Alpha cubebene, Stigmasterol and Elemol, were also projected as promising leads by the analysis.

Table 5. Obtained lead molecule using DST method.

Target Protein	Phyochemicals	AutoDock	Hex	Patch dock
Acetylcholinesterase	Betasitosterol	-6.19	-257.86	3524
Cobramine A	1 alpha epicubenol	-5.7	-170.08	2856
Cobramine B	Caryophyllene	-5.98	-183.99	2768
Cobrotoxin	Curcuminoid	-8.39	-286.53	4424
Cytotoxin	Curcuminoid	-7.35	-287.18	4124
Laminoacid oxidase	Curcuminoid	-10.32	-348.82	5918
LN1	Isochavinic acid	-5.81	-210.45	2924
LN2	Elemol	-6.14	-171.74	3122
LN3	Alpha cubebene	-5.8	-189.86	2924
LN4	Stigmasterol	-5.84	-246.55	3998
LN5	Beta Sitosterol	-7.09	-255.25	4522
PLA2	Beta Sitosterol	-11.46	-294.81	4946
Proteolase	4- Alpha methyl zymosterol	-7.17	-335.41	5774
Serine protease	Curcuminoid	-7.39	-310.81	4806

DISCUSSION

5. DISCUSSION

Snake bites are well known accidental medical emergency in all over the world especially in rural areas. The burden of snake bite envenoming still has a great impact on the population and on health care system. From the time immemorial traditional medicine recommended many plants active against different effects of snakebite . Phytochemical analysis and *in silico* validation of selected spice plants based on traditional information and their therapeutic use as medicine against insect stings and snake bites revealed that all the four plants have antidote activity. A total of 560 compounds so far reported from the spice plants such as *Allium cepa, Cinnamomum zeylanicum, Piper nigrum and Capsicum frutescence* were subjected for docking analysis. The study resulted a total of 26 compounds from the four plants as leads against 14 cobra venom proteins.

10 phytochemicals were identified as lead molecule out of 99 phytochemicals screened against fourteen cobra venom proteins . Brassicasterol in *Allium cepa* is a lead molecule against Acetyl cholinesterase and LN2; Trigonellene showed lead properties only to Cobramine B protein; 4 alpha methyl zymostenol is the lead against Cobrotoxin and L- amino acid oxidase; ; Cycloallin is the lead molecule against LN1 and LN3. Other lead molecules are 31-Norlanosterol, Allin, Caffeic acid, Beta sitosterol, 31- norcyclo artenol and Luteolin against PLA2, Proteolase, Serine protease, LN5 Cytotoxin and LN4 respectively.

The leads have been extensively reported to exhibit various pharmacological activities. Brassicasterol is a 28 carbon sterol compound suggested to reduce blood cholesterol level together with other phytosterol. Trigonelline is an alkaloid which has, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumor activities, and it has been shown to reduce diabetic auditory neuropathy and platelet aggregation. Cycloallin is an organic sulphur compound has Cardioprotective effect. Whereas Alliin, a sulfur compound has shown wide-range antifungal specificity. Luteolin is a flavanoid

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which used as an inhibitor for cell proliferation and metastasis. Caffeic acid (3,4-dihydroxycinnamic acid), one of the most common phenolic acid with several biological activities including antioxidant, anti-ischemia reperfusion, anti-thrombosis, anti-hypertension, anti-fibrosis, antivirus and antitumor properties (Jiang *et al.*,2005). β-Sitosterol a sterol compound is studied for its potential to reduce benign prostatic hyperplasia and high blood cholesterol levels.

Out of the 213 phytochemicals derived from *Piper nigrum* 9 leads were obtained against the venom proteins of Cobra. Beta cubebine inhibits 4 targets Acetyl cholinesterase, Long nuerotoxin-2, Long nuerotoxin-5 and Phospholipase-A2; Stigmasterol inhibits L-amino acid oxidase and Cytotoxin-3 similarly Isochavinic acid inhibits Long neurotoxin1& 4. Other 6 leads 1-Terpinen-4-ol, Campesterol, Cubenol, Beta sitosterol, Caffeicacid and 1-Epicubenol inhibits the activity of Long nuerotoxin-3, Cobrotoxin, Cobramine B, Proteolase, Serine protease and Cobramine A respectively.

The pharmacological importance of lead has been reported in various literatures. Beta Cubebin a volatile compound which is an active component in essential oils has been shown to possess anti-inflamatory, analgesic and trypanocidal activities. Terpene compounds Cubenol and Epi cubenol have been reported with their anti microbial activity. Stigmasterol acts as a precursor in the synthesis of progesterone and acts as an intermediate in the biosynthesis of androgens, estrogens, corticoids. The anticancer activity of Campesterol is reviewed by Awad and Fink in 2000.

Five leads obtained out of 128 phytomolecues from *Cinnamomum zeylanicum* showed inhibition properties against the total venom protein with various level of inhibition. . Curcuminoid is the best lead which could inhibit 8 out of 14 targets such as Long nuerotoxin-2, Long nuerotoxin-5, Phospholipase-A2, L-amino acid oxidase, Cytotoxin-3, Cobrotoxin, Proteolase and Serine protease. AlphaTerpineol inhibits 2 targets such as Long nuerotoxin-1&4 similarly Alphacadinol inhibits Cobramin A and B. Acetylcholineesterase and Longneurotoxin3 were inhibited by Campesterol and Piperitone respectively.

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The pharmacological importance of lead has been reported in various literatures. Curcuminoid is poly phenolic curcumin derivative has been shown to have a wide spectrum of biological actions: anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. α-Cadinol is monoterpinoid alcohol can act as anti-fungal and as hepatoprotective, and was proposed as a possible remedy for drug-resistant tuberculosis.

Twelve out of 14 venom proteins were inhibited by 8 leads screened from 120 phytochemicals derived from *Capsicum frutescence*. Solanidine inhibited Cobrotoxin and Phospholipase-A2. Solasodine inhibited Acetyl cholinesterase. Octopamine inhibited Long nuerotoxin-1,3 &4. Campesterol inhibited L-amino acid oxidase and Cytotoxin-3 similarly Malonic inhibited Serine protease and Cobramine B. Long neurotoxin-5 and Proteolase were inhibited by Caffeicacid and Quercetin respectively. The pharmacological activities of Solasodine revealed that it is an alkaloid suggested to protect cells from oxidative stress damage. Solanidine is a steroidal alkaloid which has the effects on microorganisms, cancer and inflammation. A flavanoid Quercetin has been shown to exert anticancer and anti inflammatory effects.

Strictly considering the free energy of binding as the only parameter for the selection of lead molecule, already selected leads can be inhibited more number of targets. Therefore, In *Allium cepa* , 31- Norlanostenol exhibited antitoxic activity against 7 targets, 4 alpha methyl zymostenol showed activity against 8 target proteins, , 31- Norcycloartenol showed neutralizing activity against 9 proteins and Brassicasterol showed venom neutralizing effect on 10 out of 14 cobra venom proteins. In *Piper nigrum,* Stigmasterol has activity against 3 proteins ; 1-Terpinene -4-ol and Caffeic acid has antitoxic effect on 4 proteins; Beta cubebine has free energy of binding less than -5 Kcal/mol against 6 venom proteins; While Isochavinic acid has effect on 7 proteins; Cubenol and epicubenol could inhibit 7 proteins. But Beta sitosterol and Campesterol has venom neutralizing activity

against 8 Cobra venom proteins. In *Cinnamomum zeylanicum,* Both Piperitone and Alpha terpineol showed venom neutralizing activity against 4 targets. Alpha cadinol inhibited 6 targets while Curcuminoid inhibited 9 target proteins. In *Capsicum frutescens*, Octopamine has neutralizing activity against 4venom proteins. Malonic acid and Ferulic acid showed effect on 5 proteins . Solasodine showed activity against 7 proteins. Solanidine exhibited venom neutralizing effects against 8 proteins. No phytomolecules from the plant shown binding affinity with Long nuerotoxin-2 and CobraminA.

Certain phytochemicals like Beta sitosterol, Campesterol and Alpha amyrin exhibited accepted level of free energy of binding (<= -5Kcal/mol) with majority of the target proteins . However it couldn't be considered as leads against many of the proteins as they lack enough hydrophobic and H-bond interactions. The sterol compounds Betasitosterol and Campesterol are constituents of all the selected plants. Among the studied plants, *Allium cepa* and *Capsicum frutescens* coundnt inhibit all target proteins in cobra venom, followed by *Cinnamomum zeylanicum* and *Piper nigrum* showed neutralizing activity against all targets. From these two Piper nigrum exhibited a total of 9 molecule as leads to inhibit all target whereas *Cinnamomum zeylanicum* is the plant with lesser number of leads together inhibit all the 14 toxic proteins. That is only 5 molecules were derived as leads . Curcuminoid is one of the leads here inhibit 8 target proteins. So In conclusion, it is inferred that Cinnamomum zeylanicum is the best plant for the neutralization of anticobra venom activity.

However, inorder to nullify the errors in lead identification, top ranked hit molecules were again docked using the docking tools such as Hex server and Patchdock. The docked results were statistically analysed using DST method and the selected lead molecules against target proteins were recorded . In nutshell all the four plants have significant antitoxic activity in the order of merit ranked first *Cinnamomum zeylanicum,* second *Piper nigrum,* third *Allium cepa* and the last *Capsicum frutescence.* Advance studies including *in vitro* and *in vivo* experiments with the selected lead compounds are essential to confirm the

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efficacy of the selected lead molecule and can develop them into effective drugs against cobra venom.

SUMMARY

6. SUMMARY

Snake envenomation is an important public health hazard in many regions of the globe, particularly in tropical and subtropical countries. Snakebite is considered as one of the major neglected diseases of 21st century. Snake venom is highly modified saliva that is secreted by special glands of snakes, composed of variety of bio-molecules including toxic proteins. Anti venom therapy is the only specific treatment for snake venom in modern medicine. The anti-venom immunoglobulin treatment has many limitations and side effects which include early anaphylactic reactions, pyrogenic reactions, late serum sickness type reactions, high cost, non-availability etc. Moreover, prevalent causalities occur in rural areas and agricultural fields where people are working for their livelihood. Regardless of considering all the consequences, this is the high time demand for the discovery of novel effective, cheap and better alternative drug for the management of snakebite.

Extensive literature examination revealed that there are several herbal remedies used by the ethnic people and traditional healers for the treatment of snake-bite. Spices of Kerala have their own accepted unique nutraceutical activity and have traditionally been used for the treatment of various ailments particularly as antidote against snake venom. However, the efficacy of the herbal preparations is not scientifically validated yet. Because several reasons including lack of efficient screening methods and high cost. Therefore, the present study intended to identify the active small molecule with antivenom activity from the selected spices namely *Allium cepa* L., *Capsicum fruitescens* L., *Cinnamomum zeylanicum* Blume and *Piper nigrum* L. against snake venom through *in silico* approaches.

Fourteen cobra venom toxic proteins were selected as the targets for this study. The active sites of these proteins were identified from literatures and with help of Metapocket. A total of 560 chemical molecules collected from selected spices through different literatures and databases. The canonical SMILES were retrieved from PubChem and Chemspider. The 3D structures were generated using CORINA. All the selected phytochemicals were docked into the binding

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site of each target proteins using the tool AutoDock 4.2. The docked structures having $\Delta G_{bind} \leq -5$ kcal/mol were selected as best hits and the hits were further analysed using different parameters such as number of hydrogen bonds, hydrophobic interactions, inhibition constant and Lipinski's rule of five. In order to identify the best lead, Hits were again docked using Hex and Patchdock and analysed the result using DST method.

The *in silico* analysis revealed that all the studied spices possess effective small molecules of drug value and antidote activity with varying level of binding affinity to the target venom proteins. A total of 26 leads were resulted by the study. Among them Curcuminoid, Beta sitosterol and Beta cubebine showed highly potential antitoxic effects. *Cinnamomum zeylanicum* with five active lead molecules that together inhibits all the toxic venom proteins is the best antidote spice among the four subjected for the study. *Piper nigrum* with 9 antidote lead molecules that inhibit all the toxic proteins of snake venom ranked second. The study greatly substantiates the traditional knowledge of the antidote activity of the spices of Kerala. Further *in vitro* and *in vivo* studies and preclinical/clinical trials are essential for the development of drugs out of these leads.

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APPENDIX

8. APPENDIX

Table 1. List of phytochemicals in *Allium cepa*

Sl.No.	Phytochemical	Mol. formula	Mol. wt
1.	1(f)-beta-fructosyl sucrose	$C_{18}H_{32}O_{16}$	504.43
2.	1-methyl di thio propane	C6H14S3	182.30
3.	1-o-caffeoyl-beta-d-glucose	$C_{15}H_{18}O_9$	342.29
4.	1-o-feruloyl-beta-d-glucose	$C_{16}H_{20}O_9$	356.32
5.	2,3-dimethylthiophene	C_6H_8S	112.19
6.	2,4-dimethylthiophene	C_6H_8S	112.19
7.	2,5-dimethylthiophene	C_6H_8S	112.19
8.	24-methylene-cycloartenol	$C_{31}H_{52}O$	440.74
9.	28-isofucosterol	$C_{31}H_{50}O_2$	454.72
10.	3,4-dimethyl-2,5-dioxo-2,5-dihydrothiophene	$C_6H_6O_2S$	142.17
11.	3,4-dimethylthiophene	C_6H_8S	112.19
12.	31-norcycloartenol	$C_{29}H_{48}O$	412.69
13.	31-Norlanostenol(Fdb)	$C_{29}H_{50}O$	414.70
14.	4-alpha-methyl-zymostenol	$C_{28}H_{46}O$	398.66
15.	5-Dehydroavenasterol	$C_{29}H_{46}O$	410.68
16.	9,12,13-trihydroxy-octadec-10-enoic-acid	$C_{18}H_{34}O_5$	330.45
17.	9,1o,13-trihydroxy-octadec-11-enoic-acid	$C_{18}H_{34}O_5$	330.45
18.	Ajoene	$C_9H_{14}OS_3$	234.39
19.	Allicin	$C_6H_{10}OS_2$	162.27
20.	Alliin	$C_6H_{11}NO_3S$	177.22
21.	Alliofuroside a	$C_{44}H_{72}O_{18}$	889.03
22.	Alliospiroside c	$C_{38}H_{60}O_{13}$	724.87
23.	Alliospiroside d	$C_{39}H_{62}O_{14}$	754.90
24.	Allyl-propyl-disulfide	$C_6H_{12}S_2$	148.28
25.	Alpha-amyrin	$C_{30}H_{50}O$	426.71
26.	Alpha-sitosterol	$C_{30}H_{50}O$	426.71
27.	Alpha-tocopherol	$C_{29}H_{50}O_2$	430.70
28.	Apigenin	$C_{15}H_{10}O_5$	270.24
29.	Bissulfine	$C_6H_{10}O_2S_2$	178.26
30.	Brassicasterol	$C_{28}H_{46}O$	398.67
31.	Caffeic-Acid	$C_9H_8O_4$	180.15
32.	Campesterol	$C_{28}H_{48}O$	400.68
33.	Catechol	$C_6H_6O_2$	110.11
34.	Cepanone	$C_{13}H_{22}O_2$	210.31
35.	Cholest-7-en-3-beta-ol	$C_{27}H_{46}O$	386.65
36.	Choline	$C_5H_{14}NO+$	104.17
37.	Cyanidin-3-o-beta-d-diglycoside	$C_{21}H_{21}O_{11}+$	449.38
38.	Cyanidin-3-o-laminaribioside	$C_{27}H_{31}O_{16}+$	611.52
39.	Cycloalliin	$C_6H_{11}NO_3S$	177.22
40.	Cycloartenol	$C_{30}H_{50}O$	426.71
41.	Cycloeucalenol	$C_{30}H_{50}O$	426.71
42.	Diallyl-Disulfide	$C_6H_{10}S_2$	146.27
43.	Diallyl-Sulfide	$C_6H_{10}S$	147.23

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44.	Diallyl-Trisulfide	$C_6H_{10}S_3$	178.33
45.	Dimethyl-Disulfide	$C_2H_6S_2$	94.199
46.	Dimethyl-Sulfide	C_2H_6OS	78.133
47.	Dimethyl-Trisulfide	$C_2H_6S_3$	126.26
48.	Diphenylamine	$C_{12}H_{11}N$	169.22
49.	Dipropyl-Disulphide	$C_6H_{14}S_2$	151.75
50.	Dipropyl-Trisulfide	$C_6H_{14}S_3$	182.37
51.	Eicosen-1-Ol	$C_{20}H_{40}O$	296.53
52.	Eo	$C_{20}H_{16}BrClN_2O$	415.71
53.	Ferulic acid	$C_{10}H_{10}O_4$	194.18
54.	Isoalliin	$C_6H_{11}NO_3S$	177.21
55.	Isoquercitrin	$C_{21}H_{20}O_{12}$	464.37
56.	Lutein	$C_{40}H_{56}O_2$	568.88
57.	Luteolin	$C_{15}H_{10}O_6$	286.23
58.	Melatonin	$C_{13}H_{16}N_2O_2$	232.28
59.	Methionine-Methylsulfonium	$C_6H_{14}ClNO_2S$	199.69
60.	Methionine-Sulfone	$C_5H_{11}NO_4S$	181.21
61.	Methyl-Propenyl-Trisulfide	$C_4H_8S_3$	152.30
62.	Methyl-Propyl-Disulfide	$C_4H_{10}S_2$	122.25
63.	Methylpropyl-Trisulfide	$C_4H_{10}S_3$	154.31
64.	*p*-Coumaric acid	$C_9H_8O_3$	164.16
65.	P-CYMENE	$C_{10}H_{14}$	134.22
66.	Phloroglucinol	$C_6H_6O_3$	126.11
67.	Phytosterols	$C_{29}H_{50}O$	414.70
68.	Prostaglandin E2	$C_{20}H_{32}O_5$	352.47
69.	Protocatechuic acid	$C_7H_6O_4$	154.12
70.	Pyrocatechol	$C_6H_6O_2$	110.11
71.	Quercetin	$C_{15}H_{10}O_7$	302.23
72.	Quercitrin	$C_{21}H_{20}O_{11}$	448.38
73.	Quercitrin	$C_{15}H_{10}O_7$	302.23
74.	Quinic acid	$C_7H_{12}O_6$	192.16
75.	Rutoside	$C_{27}H_{30}O_{16}$	610.52
76.	S-Allyl-Cysteine	$C_6H_{11}NO_2S$	161.22
77.	Sinapic acid	$C_{11}H_{12}O_5$	224.21
78.	S-Methyl-Cysteine	$C_4H_9NO_2S$	135.18
79.	S-Methyl-Cysteine-Sulfoxide	$C_4H_9NO_3S$	151.18
80.	Spiraeoside	$C_{21}H_{20}O_{12}$	464.37
81.	Spiraeoside	$C_{21}H_{20}O_{12}$	464.37
82.	S-Prop-1-Enyl-Cysteine-S-Oxide	$C_6H_{11}NO_3S$	177.22
83.	S-Propyl-Cysteine-Sulfoxide	$C_6H_{13}NO_3S$	179.23
84.	Stigmast-7-En-3-Beta-Ol	$C_{29}H_{50}O$	414.70
85.	Stigmasterol	$C_{29}H_{48}O$	412.69
86.	Trigonelline	$C_7H_7NO_2$	137.13
87.	TuliposideA	$C_{11}H_{18}O_8$	278.25
88.	Vanillic acid	$C_8H_8O_4$	168.14
89.	Xylitol	$C_5H_{12}O_5$	152.14
90.	Zwiebelane	$C_6H_{10}OS_2$	162.26
91.	β-sitosterol	$C_{29}H_{50}O$	414.71
92.	Stigmast-7-En-3-Beta-Ol	$C_{29}H_{50}O$	414.70
93.	Stigmasterol	$C_{29}H_{48}O$	412.69
94.	Trigonelline	$C_7H_7NO_2$	137.13
95.	TuliposideA	$C_{11}H_{18}O_8$	278.25

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Table 2. List of phytochemicals in *Piper nigrum* L.

Sl No.	Phytochemicals	Mol. formula	Mol. weight
1.	8-Trans-Piperamide-C-9-	$C_{20}H_{27}NO_3$	329.4333
2.	(-)-Cubebin	$C_{16}H_{27}NO$	356
3.	(-)-Phellandrene	$C_{10}H_{16}O$	136
4.	(+)-Limonene	$C_{10}H_{16}O$	136
5.	(Z)-Ocimenol	$C_{10}H_{16}O$	152.23
6.	1-(2,4-Dodecadienoyl)- Pyrrolidine	$C_{16}H_{27}NO$	249.39
7.	1,8(9)-P-Menthadien-4-Ol	$C_{10}H_{16}O$	152.23
8.	1,8-Cineole/Eucalyptol	$C_{10}H_{18}O$	154.24
9.	1-Alpha-Phellandrene	C10H16	136.23
10.	1-Piperyl-Pyrrolidine	$C_{16}H_{17}NO_3$	271.31
11.	1-Terpinen-4-Ol	$C_{10}H_{18}O$	154.24
12.	1-Terpinen-5-Ol	$C_{10}H_{16}O$	152.23
13.	2-Isopropyl-3-Methoxy Pyrazine	$C_{16}H_{19}NO_3$	273.32
14.	2-Methyl-Pentanoic- Acid	$C_{11}H_{22}O$	170.29
15.	2-Methyl-Pentanoic- Acid	$C_{16}H_{19}NO_3$	273.32
16.	2-Trans-Piperamide-C-5-1	$C_{11}H_{22}O$	170.2918
17.	2-Undecanone	$C_{11}H_{22}O$	170.2918
18.	3-Methyl-Butyric-Acid	$C_5H_{10}O_2$	102.13
19.	4-Methyl-Triacontane	$C_{31}H_{64}$	436.83
20.	5,10(15)-Cadinen-4-Ol	$C_{15}H_{24}O$	204.35
21.	6-Trans-Piperamide-C-7-1	$C_{18}H_{23}NO_3$	301.38
22.	Acetophenone	$C_{15}H_{24}$	204.35
23.	Acetyl-Choline	$C_7H_{16}NO_2^+$	146.20
24.	Alpha-Amorphene	$C_{15}H_{24}$	204.35
25.	Alpha-Bisabolene	$C_{15}H_{24}$	204.35
26.	Alpha-Bulnesene	$C_{15}H_{24}$	204.35
27.	Alpha-Cis- Bergamotene	C15H24	204.35
28.	Alpha-Copaene	$C_{15}H_{24}$	204.35
29.	Alpha-Cubebene	$C_{15}H_{24}$	204.35
30.	Alpha-Guaiene	$C_{15}H_{24}$	204.35
31.	Alpha-Gurjunene	$C_{15}H_{24}$	204.35
32.	Alpha-Humulene	$C_{15}H_{24}$	204.35
33.	Alpha-Linolenic-Acid	$C_{18}H_{30}O_2$	278.42
34.	Alpha-Pinene	$C_{10}H_{16}$	136.23
35.	Alpha-Santalene	$C_{15}H_{24}$	204.35
36.	Alpha-Selinene	$C_{15}H_{24}$	204.35

37.	Alpha-Terpinene	$C_{10}H_{16}$	136.23
38.	Alpha-Terpineol	$C_{10}H_{18}O$	154.24
39.	Alpha-Thujene	$C_{10}H_{16}$	136.23
40.	Alpha-Trans- Bergamotene	$C_{15}H_{24}$	204.35
41.	Alpha-Zingiberene	$C_{15}H_{24}$	204.35
42.	Arachidic-Acid	$C_{20}H_{32}O_2$	304.46
43.	Ar-Curcumene	$C_{15}H_{22}$	202.33
44.	Ascorbic-Acid	$C_6H_8O_6$	176.12
45.	Astragalin	$C_{21}H_{20}O_{11}$	448.37
46.	Azulene	$C_{15}H_{24}$	204.35
47.	Behenic-Acid	$C_{22}H_{44}O_2$	340.58
48.	Benzaldehyde	$C_{15}H_{24}$	204.35
49.	Beta-Bisabolene	$C_{15}H_{24}$	204.35
50.	Beta-Bisabolol	$C_{15}H_{26}O$	222.36
51.	Beta-Carotene	$C_{29}H_{50}O$	414.7
52.	Beta-Caryophyllene	$C_{15}H_{24}$	204.35
53.	Beta-Cubebene	$C_{15}H_{24}$	204.35
54.	Beta-Cubebine	$C_{20}H_{20}O_6$	356.36
55.	Beta-Elemene	$C_{15}H_{24}$	204.35
56.	Beta-Farnesene	$C_{15}H_{24}$	204.35
57.	Beta-Phellandrene	$C_{10}H_{16}$	136.23
58.	Beta-Pinene	$C_{10}H_{16}$	136.23
59.	Beta-Pinone	$C_9H_{14}O$	138.20
60.	Beta-Selinene	$C_{15}H_{24}$	204.35
61.	Beta-Sitosterol	$C_{29}H_{50}O$	414.70
62.	Bicyclogermacrene	$C_4H_8O_2$	88.10
63.	Borneol	$C_{10}H_{18}O$	154.24
64.	Bulnesol	$C_4H_8O_2$	88.10
65.	Butyric-Acid	$C_4H_8O_2$	88.10
66.	Caffeic-Acid	$C_9H_8O_4$	180.157
67.	Calamene	$C_{15}H_{22}$	202.33
68.	Calamenene	$C_{15}H_{22}$	202.33
69.	Campesterol	$C_{28}H_{48}O$	400.68
70.	Camphene	$C_{10}H_{16}$	136.23
71.	Capsaicin		
72.	Car-3-Ene	$C_{10}H_{16}$	136.23
73.	Carvacrol	$C_{10}H_{14}O$	150.21
74.	Carvetonacetone	C10H16O	152.23
75.	Carvone	$C_{10}H_{14}O$	150.21

76.	Carvone-Oxide	$C_{10}H_{14}O_2$	166.21
77.	Caryophellene Alcohol	$C_{15}H_{24}O$	220.35
78.	Caryophyllene	$C_{15}H_{24}$	204.35
79.	Caryophyllene-Oxide	$C_{15}H_{24}O$	220.35
80.	Cedrol	$C_{15}H_{26}O$	222.36
81.	Chavicine	$C_{17}H_{19}NO_3$	285.33
82.	Cinnamic Acid	$C_{15}H_{24}O$	220.35
83.	Cinnamic-Acid	$C_9H_8O_2$	148.15
84.	Cis P-2-Menthen-1-Ol	$C_{10}H_{14}O$	150.21
85.	Cis-2-Menthadiene-2-Ol	$C_{10}H_{14}O_2$	166.21
86.	Cis-Carveol	$C_{10}H_{16}O$	152.23
87.	Cis-Nerolidol	$C_{15}H_{26}O$	222.36
88.	Citral	$C_{10}H_{16}O$	152.2
89.	Citronellal	$C_{10}H_{18}O$	154.24
90.	Citronellol	$C_{10}H_{20}O$	156.26
91.	Citronellyl-Acetate	$C_{12}H_{22}O_2$	198.30
92.	Clovene	$C_{15}H_{24}$	204.35
93.	Cryptone	$C_9H_{14}O$	138.2
94.	Cubebol	$C_{10}H_{14}O$	150.21
95.	Cubenol	$C_{10}H_{14}O_2$	166.21
96.	Cuparene	$C_{15}H_{22}$	202.33
97.	D-Carene	$C_{10}H_{14}O$	150.21
98.	Dihydrocarveol	$C_{10}H_{18}O$	154.24
99.	Dihydrocarvone	$C_{10}H_{16}O$	152.23
100.	Dihydropipercide	$C_{21}H_{29}NO_3$	343.45
101.	Elemicin	$C_{21}H_{29}NO_3$	343.45
102.	Elemol	$C_{15}H_{26}O$	222.36
103.	Epicubenol	$C_{17}H_{21}NO_3$	287.35
104.	Epoxy Dihydro Caryophyllene	$C_{15}H_{24}$	204.35
105.	Eudesmol	$C_{15}H_{24}$	204.35
106.	Eugenol-Methyl-Ether	$C_{11}H_{14}O_2$	178.22
107.	Farnesol.	$C_{17}H_{21}NO_3$	287.35
108.	Feruperine	$C_{17}H_{21}NO_3$	287.35
109.	Gamma-Cadinene	$C_{15}H_{24}$	204.35
110.	Gamma-Muurolene	$C_{15}H_{24}$	204.35
111.	Gamma-Terpinene	$C_{10}H_{16}$	136.23
112.	Geranyl-Acetate	$C_{12}H_{20}O_2$	196.28
113.	Germacrene-B	$C_{15}H_{24}$	204.35
114.	Germacrene-D	$C_{15}H_{24}$	204.35

115.	Globulol	$C_{15}H_{26}O$	222.36
116.	Guineensine	$C_{24}H_{33}NO_3$	383.52
117.	Heliotropin	$C_8H_6O_3$	150.13
118.	Hentriacontan-16-One	$C_{31}H_{62}O$	450.82
119.	Hentriacontane	$C_{31}H_{64}$	436.83
120.	Hentriacontanol	$C_{31}H_{64}O$	452.83
121.	Heptanoate	$C_{31}H_{64}O$	452.83
122.	Hexanoic-Acid	$C_6H_{12}O_2$	116.15
123.	Hyperoside	$C_{21}H_{20}O_{12}$	464.37
124.	Iso Pinocamphone	$C_{21}H_{20}O_{12}$	464.37
125.	Isobutyl-Caproate	$C_{10}H_{20}O_2$	172.26
126.	Isobutyl-Isovalerate	$C_9H_{18}O_2$	158.23
127.	Isochavicine	$C_{17}H_{19}NO_3$	285.33
128.	Isochavinic-Acid	$C_{12}H_{10}O_4$	218.20
129.	Isoelimicin	$C_{21}H_{20}O_{12}$	464.37
130.	Isopiperine	$C_{17}H_{19}NO_3$	285.33
131.	Isopulegol	$C_{10}H_{18}O$	154.24
132.	Isoquercitrin	$C_{21}H_{20}O_{12}$	464.37
133.	Kaempferol	$C_{15}H_{10}O_6$	286.2
134.	Kaempferol-3-O- Arabinosyl-7-O-Rhamnoside	$C_{26}H_{28}O_{14}$	564.49
135.	Lauric-Acid	$C_{12}H_{24}O_2$	200.31
136.	Ledene	$C_{21}H_{20}O_{12}$	464.37
137.	Linalol/Linalool	$C_{10}H_{18}O$	154.24
138.	Linalyl-Acetate	$C_{12}H_{20}O_2$	196.28
139.	L-Limonene	$C_{10}H_{16}$	136.23
140.	Methyl Carvacrol	$C_{11}H_{14}O_2$	178.22
141.	Methyl Citronellate	$C_{21}H_{20}O_{12}$	464.37
142.	Methyl Geranate	$C_{11}H_{16}O$	164.24
143.	Methyl-Carvacrol	$C_{11}H_{16}O$	164.24
144.	Methyl-Cinnamate	$C_{10}H_{10}O_2$	162.18
145.	Methyl-Eugenol	$C_{11}H_{14}O_2$	178.22
146.	Methyl-Eugenol	$C_{10}H_{10}O_2$	162.18
147.	Methyl-Heptanoate	$C_8H_{16}O_2$	144.21
148.	Methyl-Octanoate	$C_9H_{18}O_2$	158.23
149.	M-Methyl- Acetophenone	$C_9H_{10}O$	134.17
150.	Myrcene	$C_{10}H_{16}$	136.23
151.	Myristic-Acid	$C_{14}H_{28}O_2$	228.37
152.	Myristicin	$C_{11}H_{12}O_3$	192.21

153.	Myrtenal	$C_{10}H_{14}O$	150.21
154.	Myrtenol	$C_{10}H_{16}O$	152.23
155.	N-Butyophenone	$C_{10}H_{12}O$	148.20
156.	N-Butyrophenone	$C_{11}H_{16}O$	164.24
157.	Nerol	$C_{12}H_{20}O_2$	196.28
158.	Nerol-Acetate	$C_{12}H_{20}O_2$	196.28
159.	N-Formylpiperidine	$C_6H_{11}NO$	113.157
160.	N-Hentriacontane	$C_{31}H_{64}$	436.83
161.	N-Heptadecene	$C_{17}H_{34}$	238.45
162.	Ocimene	$C_{10}H_{16}$	136.23
163.	Octanoate	$C_{12}H_{20}O_2$	196.28
164.	Oleic-Acid	$C_{18}H_{34}O_2$	282.46
165.	Oxalic-Acid	$C_2H_2O_4$	90.03
166.	Palmitic-Acid	$C_{16}H_{32}O_2$	256.42
167.	P-Coumaric-Acid	$C_9H_8O_3$	164.15
168.	P-Cymen-8-Ol	$C_{10}H_{14}O$	150.21
169.	P-Cymene	$C_{10}H_{14}$	134.21
170.	P-Cymene-8-Ol	$C_{10}H_{14}O$	150.21
171.	Pellitorine	$C_{14}H_{25}NO$	223.3544
172.	Perillaldehyde	$C_{10}H_{14}O$	150.21756
173.	Phellandral	$C_{12}H_{20}O_2$	196.286
174.	Phenylacetic-Acid	$C_8H_8O_2$	136.14792
175.	Pinocamphone	$C_{12}H_{20}O_2$	196.286
176.	Pinol	$C_{12}H_{20}O_2$	196.286
177.	Piperanine	$C_{17}H_{21}NO_3$	287.35354
178.	Piperettine	$C_{19}H_{21}NO_3$	311.37494
179.	Piperic Acid	$C_{12}H_{10}O_4$	218.2054
180.	Pipericine	$C_{22}H_{41}NO$	335.567
181.	Piperidine	$C_5H_{11}N$	85.14754
182.	Piperine	$C_{17}H_{19}NO_3$	285.33766
183.	Piperitone	$C_{10}H_{16}O$	152.23344
184.	Piperolein-A	$C_{19}H_{25}NO_3$	315.4067
185.	Piperolein-B	$C_{21}H_{29}NO_3$	343.45986
186.	Piperonal	$C_8H_6O_3$	150.13144
187.	Piperonic Acid	$C_{12}H_{20}O_2$	196.286
188.	P-Menth-8-En-1-Ol	$C_{10}H_{18}O$	154.24932
189.	P-Methyl- Acetophenone	$C_9H_{10}O$	134.1751
190.	Pyrrolidine	C_4H_9N	71.12096

191.	Pyrroperine/Piperyline	$C_{16}H_{17}NO_3$	271.3111
192.	Quercetin	$C_{15}H_{10}O_7$	302.2357
193.	Quercitrin	$C_{21}H_{20}O_{11}$	448.3769
194.	Retrofractamide-A/ Pipercide	$C_{20}H_{25}NO_3$	327.4174
195.	Rhamnetin	$C_{16}H_{12}O_7$	316.26228
196.	Rutin	$C_{27}H_{30}O_{16}$	610.5175
197.	Sabinene	$C_{10}H_{16}$	136.23404
198.	Safrole	$C_{10}H_{10}O_2$	162.1852
199.	Sarisan	$C_{12}H_{20}O_2$	196.286
200.	Sesquisabinene	$C_{15}H_{24}$	204.35106
201.	Sesquiterpene	$C_{12}H_{20}O_2$	196.286
202.	Spathulenol	$C_{15}H_{24}O$	220.35046
203.	Terpenolene Epoxide	$C_{12}H_{20}O_2$	196.286
204.	Terpin-1-En-4-Ol	$C_{10}H_{18}O$	154.24932
205.	Terpinolene	$C_{10}H_{16}$	136.23404
206.	T-Muurolol	$C_{12}H_{20}O_2$	196.286
207.	Trans Limonene Epoxide	$C_{12}H_{20}O_2$	196.286
208.	Trans-Carveol	$C_{10}H_{16}O$	152.23344
209.	Trans-Nerolidol/Nerolidol	$C_{15}H_{26}O$	222.36634
210.	Trans-Pinocarveol	$C_{10}H_{16}O$	152.23344
211.	Trichostachine	$C_{16}H_{17}NO_3$	271.31108
212.	Trns Anethol	$C_{12}H_{20}O_2$	196.286
213.	Virdiflorol	$C_{16}H_{17}NO_3$	271.31108

Table 3. List of phytochemicals in *Cinnamomum zeylanicum*

Sl. No.	Phytochemicals	molecular formula	molecular weight(Da)
1.	(-)-Epicatechin	$C_{15}H_{14}O_6$	290.271
2.	(E)(E)-Farnesol	$C_{15}H_{26}O$	222.372
3.	(E)-Beta-Ocimene	C10H16	136.238
4.	(E)-Methyl-Isoeugenol	$C_{11}H_{14}O_2$	178.231
5.	(Epi-A) Bisabolol	$C_{15}H_{26}O$	222.372
6.	(Epi-A) Cadinol	$C_{15}H_{26}O$	222.372
7.	(Z)-Beta-Ocimene	$C_{10}H_{16}$	136.238
8.	(Z)-Ocimenol	$C_{10}H_{18}O$	154.253
9.	(Z,E)-Alpha-Farnesene	$C_{15}H_{24}$	204.357
10.	1,8-Cineole	$C_{10}H_{18}O$	154.253
11.	2-Phenylacetaldehyde	C_8H_8O	120.151
12.	2-Phenylethanol	$C_8H_{10}O$	122.167
13.	2-Phenylethylbenzoate	$C_{15}H_{14}O_2$	226.275
14.	2-Phenylethylpropionate	$C_{11}H_{14}O_2$	178.231
15.	2-Propenal,3-2 Methoxyphenyl	$C_{10}H_{10}O_2$	162.1852
16.	2-Vinylphenol	C_8H_8O	120.151
17.	3,4-Dihydroxybenzoic Acid	$C_7H_6O_4$	154.121
18.	3-Phenylpropanal	$C_9H_{10}O$	134.178
19.	3-PHENYL-Propylacetate	$C_{11}H_{14}O_2$	178.231
20.	Acetoeugenol	$C_{12}H_{14}O_3$	206.241
21.	Acetyl-Eugenol	$C_{12}H_{14}O_3$	206.241
22.	Alpha-Humulene	$C_{15}H_{24}$	204.357
23.	Alpha-Phellandrene	$C_{10}H_{16}$	136.238
24.	Alpha-Pinene	$C_{10}H_{16}$	136.238
25.	Alpha-Terpinene	$C_{10}H_{16}$	136.238
26.	Alpha-Terpineol	$C_{10}H_{18}O$	154.253
27.	Alpha-Thujene	$C_{10}H_{16}$	136.238
28.	Alpha-Ylangene	$C_{15}H_{24}$	204.357
29.	Ar-Curcumene	$C_{15}H_{22}$	202.341
30.	Benzenepropanol	$C_9H_{12}O$	136.194
31.	Beta-Caryophyllene	$C_{15}H_{24}$	204.357
32.	Beta-Elemene	$C_{15}H_{24}$	204.357
33.	Beta-Selinene	$C_{15}H_{24}$	204.357
34.	Borneol	$C_{10}H_{18}O$	154.253
35.	Borneol-Acetate	$C_{12}H_{20}O_2$	196.29
36.	Bornyl-Acetate	$C_{12}H_{20}O_2$	196.29
37.	Cadalene	$C_{15}H_{18}$	198.309
38.	Caffeic-Acid	$C_9H_8O_4$	180.159

39.	Campesterol	$C_{28}H_{48}O$	400.691
40.	Camphene	$C_{10}H_{16}$	136.238
41.	Camphor	$C_{10}H_{16}O$	152.237
42.	Car-3-Ene	$C_{10}H_{16}$	136.238
43.	Caryophyllene Oxide	$C_{15}H_{25}$	220.356
44.	Catechins	$C_{15}H_{14}O_6$	290.271
45.	Cinnamaldehyde	C_9H_8O	132.148
46.	Cinnamic Acid	$C_9H_8O_2$	148.161
47.	Cinnamic-Acid-Ethylester	$C_{11}H_{12}O_2$	176.215
48.	Cinnamtannin B1	$C_{45}H_{36}O_{18}$	864.765
49.	Cinnamyl Acetate	$C_{11}H_{12}O_2$	176.215
50.	Cinnamyl Alcohol	$C_9H_{10}O$	134.178
51.	Cinnzeylanin	$C_{22}H_{34}O_8$	426.506
52.	Cinnzeylanol	$C_{20}H_{32}O_7$	384.469
53.	CIS-Linalool-OXIDE	$C_{10}H_{18}O_2$	170.252
54.	Citronellal	$C_{10}H_{18}O$	154.253
55.	Coniferaldehyde	$C_{10}H_{18}O$	154.253
56.	Coumarin	$C_{15}H_{26}$	146.145
57.	Cumene	C_9H_{12}	120.195
58.	Cuminaldehyde	$C_{10}H_{12}O$	148.205
59.	Curcuminoid	$C_{15}H_{28}$	424.409
60.	Cyanidin	$C_{15}H_{11}O_6^+$	287.247
61.	Delta-3-Carene	$C_{10}H_{16}$	136.238
62.	Dihydrocinnamaldehyde	$C_9H_{10}O$	134.178
63.	Dihydrofumigatin	$C_8H_{10}O_4$	170.164
64.	E Cinamyl Acetate	$C_{11}H_{12}O_2$	176.215
65.	E Cinnamaldehyde	C_9H_8O	132.162
66.	Estragole	$C_{10}H_{12}O$	148.205
67.	Ethyl-Cinnamate	$C_{11}H_{12}O_2$	176.215
68.	Eugenol	$C_{15}H_{27}$	164.204
69.	Eugenol	$C_{10}H_{12}O_2$	164.204
70.	Eugenol-Acetate	$C_{12}H_{14}O_3$	206.241
71.	Eugenol-Methyl-Ether	$C_{11}H_{14}O_2$	178.231
72.	Farnesol	$C_{15}H_{26}O$	222.372
73.	Fenchone	$C_{10}H_{16}O$	152.237
74.	Flavan-3-Ol	$C_{15}H_{14}O_2$	226.275
75.	Furfural	$C_5H_4O_2$	96.085
76.	Furfurol	$C_5H_6O_2$	98.101
77.	Gamma-Cadinene	$C_{15}H_{24}$	204.357
78.	Gamma-Terpinene	$C_{10}H_{16}$	136.238
79.	Gamma-Terpineol	$C_{10}H_{18}O$	154.253

80.	Geranial	$C_{10}H_{16}O$	152.237
81.	Geraniol	$C_{10}H_{18}O$	154.253
82.	Geraniol-Acetate	$C_{12}H_{20}O_2$	196.29
83.	Hydrocinnamaldehyde	$C_9H_{10}O$	134.178
84.	Isocaryophyllene	$C_{15}H_{24}$	204.357
85.	Limonene	$C_{10}H_{16}$	136.238
86.	Linalool	$C_{10}H_{18}O$	154.253
87.	Linalool	$C_{10}H_{18}O$	154.253
88.	Linalyl-Acetate	$C_{12}H_{20}O_2$	196.29
89.	Mannitol	$C_6H_{14}O_6$	182.172
90.	Methyl Cinnamate	$C_{10}H_{10}O_2$	162.188
91.	Methyl-Cinnamate	$C_{10}H_{10}O_2$	162.188
92.	Methyl-N-Amylketone	$C_7H_{14}O$	114.188
93.	Methyl-Vinyl-Ketone	C_4H_6O	70.091
94.	Myrcene	$C_{10}H_{16}$	136.238
95.	Nerol	$C_{10}H_{18}O$	154.253
96.	N-Octanal	$C_8H_{16}O$	128.215
97.	Nonan-1-Al	$C_9H_{18}O$	142.242
98.	Omethoxycinnamaldehyde	$C_{10}H_{10}O_2$	162.188
99.	Omethoxycinnamaldehyde	$C_{10}H_{10}O_2$	162.188
100.	Oxalic-Acid	$C_2H_2O_4$	90.034
101.	P-Coumaric-Acid	$C_9H_8O_3$	164.16
102.	P-Cymene	$C_{10}H_{14}$	134.222
103.	Phellandrene	$C_{10}H_{16}$	136.238
104.	Phytosterols	$C_{29}H_{50}O$	414.718
105.	Piperitone	$C_{10}H_{16}O$	152.237
106.	Proanthocyanidin	$C_{31}H_{28}O_{12}$	592.553
107.	Proanthocyanidin-A-2	$C_{30}H_{24}O_{12}$	576.51
108.	Proanthocyanidin-B1	$C_{30}H_{26}O_{12}$	578.526
109.	Proanthocyanidin-B2	$C_{30}H_{26}O_{12}$	578.526
110.	Proanthocyanidin-B5	$C_{30}H_{26}O_{12}$	578.526
111.	Proanthocyanidin-C1	$C_{45}H_{38}O_{18}$	866.781
112.	Quercetin-3-*O*-A-L-Rhamnopyranoside	$C_{21}H_{20}O_{11}$	448.38
113.	Rutin	$C_{27}H_{30}O_{16}$	610.521
114.	Sabinene	$C_{10}H_{16}$	136.238
115.	Safrole	$C_{10}H_{10}O_2$	162.188
116.	Salicylates	$C_7H_5O_3$-	137.114
117.	Terpin-4-EN-1-OL	$C_{10}H_{18}O$	154.253
118.	Terpinolene	$C_{10}H_{16}$	136.238
119.	Tetradecan-1-Al	$C_{14}H_{28}O$	212.377
120.	Trans Calamemene	$C_{15}H_{22}$	202.341

121.	*Trans*-A-Bergamotene	$C_{15}H_{24}$	204.357
122.	Urolignoside	$C_{26}H_{34}O_{11}$	522.547
123.	Vanillin	$C_8H_8O_3$	152.149
124.	Z Cinnamaldehyde	C_9H_8O	132.162
125.	A Copaene	$C_{15}H_{24}$	204.357
126.	A Muurolene	$C_{15}H_{24}$	204.357
127.	*A*-Muurolol	$C_{15}H_{26}O$	222.372
128.	ϒ Muurolene	$C_{15}H_{24}$	204.357

Table 4. List of phytochemicals in *Capsicum frutescens.*

Sl. No.	Phytochemicals	Molecular Formula	Molecular Weight
1.	1,8-Cineole	$C_{18}H_{29}NO_3$	307.434
2.	2-Decenoic-Acid	$C_{10}H_{18}O$	154.253
3.	2-Heptanone	$C_{10}H_{18}O_2$	170.252
4.	2-Iso-Butyl-3-Methoxypyrazine	$C_7H_{14}O$	114.188
5.	2-Methoxy-3-Isobutylpyrazine	$C_9H_{14}N_2O$	166.220
6.	2-Methyl-Butanal	$C_9H_{14}N_2O$	166.224
7.	2-Methyl-Butyric-Acid	$C_5H_{10}O$	86.134
8.	2-Octanone	$C_5H_{10}O_2$	102.133
9.	2-Octenoic-Acid	$C_8H_{16}O$	128.215
10.	2-Pentyl-Furan	$C_8H_{14}O_2$	142.198
11.	2-Undecanone	$C_9H_{14}O$	138.21
12.	3-Acetamido-2-Methylpentadecane	$C_{11}H_{22}O$	170.296
13.	3-Methyl-Butanal	$C_5H_{12}O$	88.15
14.	4-Methyl-3-Penten-2-One	$C_5H_{10}O$	86.134
15.	4-Methyl-Heptadecane	$C_6H_{10}O$	98.145
16.	4-Methyl-Hexadecane	$C_{18}H_{38}$	254.494
17.	4-Methylpentadecane	$C_6H_{12}O_2$	116.16
18.	4-Methyl-Pentan-1-Ol	$C_{17}H_{36}$	240.475
19.	4-Methyl-Pentanoicacid	$C_6H_{14}O$	102.177
20.	4-Methylpentyl-2-Methyl-Butyrate	$C_{16}H_{34}$	226.448
21.	5-Methyl-2-Furfural	$C_{15}H_{32}$	212.421
22.	8-Methyl-Nonanoicacid	$C_{10}H_{19}O_2$	171.257
23.	Alpha-Linolenic-Acid	$C_5H_{11}NO_2$	117.148
24.	Alpha-Phellandrene	$C_{40}H_{56}$	536.888
25.	Alpha-Terpineol	$C_{10}H_{16}$	136.238
26.	Antheraxanthin	$C_{10}H_{18}O$	154.253
27.	Apiin	$C_{40}H_{56}O_3$	584.885
28.	Aurochrome	$C_{26}H_{28}O_{14}$	564.496
29.	Benzaldehyde	$C_{40}H_{56}O_2$	568.886
30.	Beta-Caroteneepoxide	C_7H_6O	106.124
31.	Betaine	$C_{10}H_{16}$	136.238
32.	Beta-Ionone	$C_{13}H_20O$	191.3
33.	Betaphenethylacetate	$C_{13}H_{20}O$	192.302
34.	Beta-Pinene	C10H12O2	164.204
35.	Campesterol	$C_{18}H_{30}O_2$	278.436
36.	Camphor	$C_{28}H_{48}O$	400.691
37.	Capsaicin	$C_{10}H_{16}O$	152.237
38.	Capsanthin	$C_{18}H_{27}NO_3$	305.418
39.	Capsanthin-3,6-Epoxide	$C_{40}H_{56}O_3$	584.885
40.	Capsanthin-5,6-Epoxide	$C_{40}H_{56}O_4$	600.87
41.	Capsanthone	$C_{40}H_{56}O_4$	600.8702
42.	Capsiamide	$C_{40}H_{54}O_3$	582.869
43.	Capsicin	$C_{18}H_{27}NO_3$	305.418
44.	Capsidiol	$C_{17}H_{35}NO$	269.473
45.	Capsolutein	$C_{15}H_{24}O_2$	236.355
46.	Carnaubic-Acid	$C_{40}H_{56}O_4$	600.884

47.	Caryophyllene	$C_{40}H_{56}O_4$	600.884
48.	Chlorogenic-Acid	$C_{15}H_{24}$	204.357
49.	Cinnamic-Acid	$C_{16}H_{18}O_9$	354.311
50.	Cis-3-Hexen-1-Ol	$C_9H_8O_2$	148.161
51.	Citroxanthin	$C_6H_{12}O$	100.161
52.	Citrullin	$C_{40}H_{56}O$	552.887
53.	Cryptocapsin	$C_6H_{13}N_3O_3$	175.188
54.	Cryptoxanthin	$C_{40}H_{56}O_2$	568.886
55.	Cucurbitaxanthin-A	$C_{40}H_{56}O$	552.887
56.	Cucurbitaxanthin-B	$C_{40}H_{56}O_3$	584.885
57.	Cycloviolaxanthin	$C_{40}H_{56}O_4$	600.884
58.	Decanoic-Acidvanillylamide	$C_{40}H_{56}O_4$	600.884
59.	Delta-3-Carene	$C_{10}H16$	136.238
60.	Dihydrocapsaicin	$C_{18}H_{29}NO_3$	307.434
61.	Dihydrocapsicin	$C_{18}H_{27}NO_3$	305.418
62.	Eriodictin	$C_{21}H_{22}O_{10}$	434.397
63.	Ferulic-Acid	$C_{10}H_{10}O_4$	194.186
64.	Foliaxanthin	$C_{40}H_{56}O_4$	600.884
65.	Geranyl-Acetone	$C_{13}H_{22}O$	194.318
66.	Heneicosane	$C_{21}H_{44}$	296.583
67.	Heptadecane	$C_{17}H_{36}$	240.475
68.	Heptanoic-Acid	$C_7H_{14}O_2$	130.187
69.	Hesperidin	$C_{28}H_{34}O_{15}$	610.565
70.	Hexadecane	$C_{16}H_{34}$	226.448
71.	Hexanoic-Acid	$C_6H_{12}O_2$	116.16
72.	Homocapsaicin	$C_{19}H_{29}NO_3$	319.445
73.	Homodihydrocapsaicin	$C_{19}H_{31}NO_3$	321.461
74.	Homodihydrocapsaicin-1	$C_{19}H_{31}NO_3$	321.461
75.	Isobutyric-Acid	$C_4H_8O_2$	88.106
76.	Isohexanoic-ACID	$C_6H_{12}O_2$	116.16
77.	Isohexyl-Isocaproate	$C_{12}H_{24}O_2$	200.322
78.	Isothujone	$C_{10}H_{16}O$	152.237
79.	Isovaleric-Acid	$C_5H_{10}O_2$	102.133
80.	Kaempferol	$C_{15}H_{10}O_6$	286.239
81.	Latoxanthin	$C_{40}H_{58}O_5$	618.899
82.	Limonene	$C1_0H_{16}$	136.238
83.	Lutein	$C_{40}H_{56}O_2$	568.886
84.	Luteolin-7-Monoglucoside	$C_{21}H_{20}O_{11}$	448.38
85.	Malonic-Acid	$C_3H_4O_4$	104.061
86.	Margaric-Acid	$C_{17}H_{34}O_2$	270.457
87.	Methyl-Decanoate	$C_{11}H_{22}O_2$	186.295
88.	Methyl-Dodecanoate	$C_{13}H_{26}O_2$	214.349
89.	Methyl-Heptanoate	$C_8H_{16}O_2$	144.214
90.	Methyl-Hexanoate	$C_7H_{14}O_2$	130.187
91.	Methyl-Nonanoate	$C_{10}H_{20}O_2$	172.268
92.	Methyl-Octanoate	$C_9H_{18}O_2$	158.241
93.	Methyl-Pentanoate	$C_6H_{12}O_2$	116.16
94.	Methyl-Phenylacetate	$C_9H_{10}O_2$	150.177
95.	Mevalonic-Acid	$C_6H_{12}O_4$	148.158
96.	Myrcene	$C_{10}H_{16}$	136.238

97.	N-(13)-Methyltetradecyl)Acetamide	$C_{17}H_{35}NO$	269.473
98.	Neoxanthin	$C_{40}H_{56}O_4$	600.884
99.	N-Hexanal	$C_6H_{12}O$	100.161
100.	Nigroxanthin	$C_{40}H_{54}O$	550.871
101.	Nonadecane	$C_{19}H_{40}$	268.529
102.	Nonanoic-Acid	$C_9H_{18}O_2$	158.241
103.	Norcapsaicin	$C_{17}H_{27}NO_3$	291.391
104.	Nordihydrocapsaicin	$C_{17}H_{27}NO_3$	293.407
105.	Novivamide	$C_{17}H_{27}NO_3$	293.407
106.	Octanoic-Acid	$C_8H_{16}O_2$	144.214
107.	Octopamine	$C_8H_{11}NO_2$	153.181
108.	Oxalic-Acid	$C_2H_2O_4$	90.034
109.	P-Coumaric-Acid	$C_9H_8O_3$	164.16
110.	Pentadecane	$C_{15}H_{32}$	212.421
111.	Pentadecanoic-Acid	$C_{15}H_{30}O_2$	242.403
112.	Pentanoic-Acid	$C_5H_{10}O_2$	102.133
113.	Phylloquinone	$C_{31}H_{46}O_2$	450.707
114.	Phytosterols	$C_{29}H_{50}O$	414.718
115.	P-Methylacetophenone	$C_9H_{10}O$	134.178
116.	Pulegone	$C_{10}H_{16}O$	152.237
117.	P-Xylene	$C_6H_4(CH_3)_2$	106.168
118.	Quercetin	$C_{15}H_{10}O_7$	302.238
119.	Rutin	$C_{27}H_{30}O_{16}$	610.521
120.	Scopoletin	$C_{10}H_8O_4$	192.17
121.	Solanidine	$C_{27}H_{43}NO$	397.647
122.	Solanine	$C_{45}H_{73}NO_{15}$	868.071
123.	Solasodine	$C_{27}H_{43}NO_2$	413.646
124.	Terpinen-4-Ol	$C_{10}H_{18}O$	154.253
125.	Tetradecane	$C_{14}H_{30}$	198.394
126.	Thujone	$C_{10}H_{16}O$	152.237
127.	Trans-2-Hexen-1-Ol	$C_6H_{12}O$	100.161
128.	Valeric-Acid	$C_5H_{10}O_2$	102.133
129.	Vanillyl-Amine	$C_8H_{11}NO_2$	153.181
130.	Violaxanthin	$C_{40}H_{56}O_4$	600.884
131.	Zucapsaicin	$C_{40}H_{56}O_2$	568.886

"IDENTIFICATION OF LEAD COMPOUNDS WITH ANTI- COBRA VENOM ACTIVITY IN COMMON SPICES THROUGH *IN SILICO* METHODS"

By

RAHUMATH N.

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Abstract of Thesis

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9. ABSTRACT

Snake bite envenomation a major neglected tropical disease, is a medical emergency in 21st century. Antisnake venom therapy, the only specific antidote to snake venom has many limitations and side effects. To overcome the backdrops, discovery of novel faster and better alternative drug is necessary. Since time immemorial several herbal remedies have been used against snake bite by traditional healers. The present study aims to scientifically validate the efficacy of phytochemicals reported from three spice plants of Kerala namely *Allium cepa* L., *Capsicum fruitescens* L., *Cinnamomum zeylanicum* Blume and *Piper nigrum* L. against selected 14 cobra venom toxic proteins using *in silico* approaches. Total 560 phytochemicals were screened against the target toxic proteins. The docked structures having free energy of binding less than -5 kcal/mol were selected as best hits and further analysis were done with the parameters such as hydrogen bonds, hydrophobic interaction, inhibition constant and Lipinski's rule of five. Comparative analysis of hits from total phytochemicals was done using DST method. The results revealed that all the spices studied possess effective small molecules of drug value and antidote activity with varying level of binding affinity to the target venom proteins. A total of 26 leads were resulted by the study. Among them Curcuminoid, Beta sitosterol and Beta cubebine showed highly potential antitoxic effects. *Cinnamomum zeylanicum* with five active lead molecules that together inhibits all the toxic venom proteins is the best antidote spice among the four subjected for the study. *Piper nigrum* with 9 antidote lead molecules that inhibit all the toxic proteins of snake venom ranked second. The study greatly substantiates the traditional knowledge of the antidote activity of the spices of Kerala. Further *in vitro* & *in vivo* studies and preclinical/clinical trials are essential for the development of drugs out of these leads.