In silico screening and molecular docking of phytochemical compounds to identify novel mosquito/insect repellent compounds targeting the odorant binding proteins (OBPs) of *Anopheles gambiae* and *Anopheles stephensi*

A

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IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN BIOINFORMATICS

By

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CERTIFICATE-II

This is to certify that the dissertation entitled ***"In silico screening and molecular docking of phytochemical compounds to identify novel mosquito/insect repellent compounds targeting the odorant binding proteins (OBPs) of Anopheles gambiae and Anopheles stephensi"*** submitted by **Miss Swati Sucharita Satpathy** to the Orissa University of Agriculture & Technology, Bhubaneswar in the partial fulfilment of the requirements for the award of the degree of **Master of Science** in **Bioinformatics** has been approved by the students advisory committee after an oral examination of the same in collaboration with external examiner.

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Date:…… **Ms Swati Sucharita Satpathy**

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ABBREVIATION

BLAST	:	Basic Local Alignment Search Tool
DEET	:	N, N-Diethyl-3-methylbenzamide
DSV	:	Discovery Studio Visualizer4.0
IRS	:	Indoor Residual Spray
I-TASSER	:	Iterative Threading ASSEmbly Refinement
NVBDCP	:	National Vector Borne Disease Control Programme
OBP	:	Odorant Binding Protein
OR	:	Odorant receptors
ORN	:	Olfactory receptor neuron
PBP	:	Pheromone Binding Proteins
PMD	:	p-menthane 3, 8-diole
RMSD	:	root-mean-square deviation
VBD	:	Vector-borne diseases
WHO	:	World Health Organization

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Title of Thesis	***In silico* screening and molecular docking of phytochemical compounds to identify novel mosquito/insect repellent compounds targeting the odorant binding proteins (OBPs) of *Anopheles gambiae* and *Anopheles stephensi***
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ABSTRACT

Vector borne diseases (VBD) such as malaria, dengue fever, plague, yellow fever, sleeping sickness, Leishmaniasis, West Nile encephalitis, *Japanese encephalitis*(JE) etc. are one of the largest threats to mankind. The vectors i.e., mosquitoes and ticks are the transmitters of disease-causing parasites and carry the pathogens from one host to another. It has been estimated that more than millions of people residing in the tropical regions of Africa, Asia and South America, lose their lives due to VBD every year. Further, about 1.4 million are attributed to VBD, out of 11 million deaths due to various infectious diseases. Significant volume of efforts have been devoted towards the development of efficient insecticides or insect repellents. Biodefense strategy (i.e., larvicides and larvivorous fish) along with chemical methods have been employed to control VBD, which were found to be more expensive and time consuming. Odorant binding proteins (OBPs) are considered as important targets for structure-based rational approaches for the discovery of new repellent or other olfaction inhibitory compounds with desirable features. But, little effort has been made to screen phyto-chemicals compounds with desired activity for the design of novel mosquito/insect repellents using high-throughput computational biology tool is scanty. Henceforth, in this study, we made an attempt to screen phytochemicals from 10 plants with mosquito repellent activity from published literature and public domain through theoretical modelling and molecular docking studies targeting odorant binding proteins (OBPs) of *Anopheles gambiae* and *Anopheles stephensi* (vectors of malaria). The widely used mosquito repellent N, N-Diethyl-m-toluamide (DEET) was selected as reference to check the binding affinity and specificity of the compounds against OBPs. A total of 40 compounds and DEET

was docked to the active site of OBP models/crystal structures using the AutoDockv4.2.6. Among these phytochemical compounds, a total of 17 compounds showed higher binding energy and higher number of hydrogen-bonds as compared to DEET. Few compounds identified in this study *i.e.*, azadirachtin, lycopersin, khusimol, khusimone and alpha-vertivone showing high docking scores needs further investigation which may aid in the design of safer and more effective insect repellents. The results from the present study is expected to steer the process of discovery of novel and effective repellent which will open better avenues to bring new possibilities in limiting the threat of malaria in near future.

Key words: DEET, phytochemicals, sesquiterpenes, odorant-binding protein.

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CHAPTER I

Introduction

Vector-borne diseases are illnesses that are transmitted by vectors, which include mosquitoes, ticks, and fleas. These vectors can carry infective pathogens such as viruses, bacteria, and protozoa, which can be transferred from one host (carrier) to another. A number of potentially lethal diseases, including encephalitis, yellow fever and malaria, are transmitted by blood feeding insects. Mosquitoes act as vector for many protozoans, bacterial and viral diseases (Service, 1996). Increase in mosquito population is a perennial problem for many developing countries due to spread of diseases like malaria, filarial, encephalitis etc. Amongst them, the most deadly tropical disease is malaria. Malaria is transmitted to humans by female mosquitoes, particularly *Anopheles* species, carrying the pathogen parasite *Plasmodium falciparum* in their saliva. Mosquito is the most indisputable medicinal significant arthropod vector of diseases. The vector-borne diseases caused by mosquito are one of the major health problems in most of the countries.

To protect humans against mosquito borne diseases, mosquito repellents' in different forms (herbs, aromatic oils and synthetic compounds) were widely used all over world. Disruption of the normal olfactory responses to odor molecules that control mosquito behavior provides a means to reduce human-mosquito interactions; a primary method for achieving this is the use of repellents. N, N-Diethyl-3-methylbenzamide (DEET) is considered the most broad spectrum and effective insect repellent available (Katz, 2008). Out of many available synthetic mosquito repellents, the best known chemical insect repellent is DEET (Fradin and Day, 2002). It will be more surprising to know that over 40 years of its discovery and usage, DEET gain remarkable safety profile (Fradin, 1998). DEET shows highest binding inhibition rate (88.7–92.5 %) against wide range of mosquitoes. It has been shown that DEET blocks electro physiological responses of olfactory sensory neurons to odors in *Anopheles* (Ditzen, 2008). Alternatively, it has been proposed that DEET functions to activate olfactory neurons that elicit avoidance behavior (Syed, 2008). Recent studies have provided evidence that DEET can also interact directly with OBPs, including OBP1 (Tsitsanou, 2012).

However, several studies have shown that the use of synthetic mosquito repellents at faster pace could cause many side effects (slurred speech, muscle twisting, seizures, rashes, vomiting

and nausea) and elevate issues concerning human health. To safeguard human health, use of safe natural compounds from plant extracts became an alternative approach to minimize the side effects as compared to synthetic mosquito repellents. Historically, it is well known that plant extracts were extensively used as potential natural repellents against wide range of insects over 2000 years ago (Nentwing, 2003). Moreover, many natural herbs have been evaluated for their flavorings characters and therapeutic properties (Singh *et al*., 2010). Since ages, ancient people use herbal plants in different ways to repel mosquitoes. Widely used practices include generation of smoke by burning plants (Sharma and Ansari, 1993), hanging fresh plants in houses for avoiding mosquitoes in the near vicinity (Waka *et al*., 2006). In contrast to whole herbs, studies confirm that plant extracts form wide range of plants are more advantageous and efficacious.

However, the newly discovered natural compounds from potential medicinal plants have not been fully explored due to their toxic characteristics (Metacalf 1962; Pan *et al*., 2013; Abd Kadir *et al.*, 2013; Grzybowski *et al*., 2011; Atanasov *et al*., 2015). Recent studies have shown that the plant extracts shows good larvicidal activity against mosquitoes. Several studies have also confirmed that plant extracts from *Phytolacca dodencandra* (pokeweed) showed larvicidal activity against mosquitoes (Dahlman and Hibb 1967, Owiti *et al*., 2015; Getachew *et al*., 2016).

According to the World Health Organization (WHO), global climate change is expanding mosquitoes range, heightening the risk of disease for millions of additional people (WHO, 1996). Primary prevention is one of the most important aspects to subside the spread of diseases either by controlling the population of these vectors or by preventing the interaction between the vector and the host. The *Anopheles gambiae* mosquito, which is the vector for *Plasmodium falciparum* malaria, uses a series of olfactory cues emanating from human sweat to select humans as their source for a blood meal. Perception of these odors within the mosquito olfactory system involves the interplay of odorant-binding proteins (OBPs) and odorant receptors and disrupting the normal responses to those odorants that guide mosquito-human interactions represents an attractive approach to prevent the transmission of malaria (Emma and Murphy, 2012).

Repellents play an important role in vector control and prevention, particularly in those areas where the biology and feeding behavior or mosquito vectors are less favorable to existing or available methods (Bellini, 2014). Olfactory stimulus plays a major role in insect behaviors such as host-seeking, oviposition, and mating. For example, female *Anopheles* mosquitoes, which are

the main vectors of malaria transmission, use olfactory cues to find human hosts and avoid non-human hosts (Pates, 2001).

Female mosquitoes of the *Anopheles* species are the primary vector for transmission of *Plasmodium falciparum* malaria and are attracted to humans by odors that emanate from incubated human sweat (Braks, 1999). Olfaction is a finely tuned sense, able to detect and discriminate between thousands of volatile molecules at low concentrations, some differing by only one or a few atoms. Olfaction in insects relies on dedicated organs in the antennae, which gather the olfactory neurons located at the base of a large number of sensilla (Krieger, 1997). Each sensillum can be considered as an elementary module possessing all of the components necessary for translating a chemical signal into an electrical stimulus (Sato, 2008). Understanding the molecular mechanism for human host recognition mediated by olfaction would help in identifying new strategies for the prevention of the primary contact. Volatile products secreted by the human host in the process of metabolism are responsible for the attraction of these vectors to the host. The ability of recognizing and discriminating thousands of odorant molecules in insects as in mammals relies on specialized chemo sensitive neural cells expressing olfactory receptor proteins (ORs) which reside within segregated compartments called sensilla. Each sensillum is a hair-like structure bathed in the sensillum lymph which contains a number of secreted proteins (McKenna *et al*., 1994; Pikielny *et al*., 1994; Wang *et al*., 1999). The odorant binding proteins (OBPs) are found to be important water-soluble components of this sensillum lymph. Lots of sensilla are located on the surface of the insect antennae with olfactory neurons being protected inside. There are 1-4 dendrites per olfactory sensilla and they are immersed in the hemolymph.

Therefore, to trigger the olfactory signal transduction, ligands in the habitat should pass through the hemolymph to stimulate specific dendrites. It was first identified in the moth as pheromone binding proteins (PBPs) (Vogt and Riddiford, 1981). These globular proteins are believed to bind different odorant molecules (Plettner *et al*., 2000), owing to their high divergence within the family, and transport them to their respective olfactory receptors triggering the mechanism of olfaction (Pelosi and Maida, 1995). The OBPs form a large specific multi-gene family. They are 10–30 kDa globular and water-soluble proteins that are characterized by a specific six a-helical domain comprising of six highly conserved cysteines that have distinct disulphide connectivities. These structural features are now considered the hallmark of this protein family (Calvo *et al*., 2002; Valenzuela *et al*., 2002; Calvo *et al*., 2006).

Indeed, insects have diverse olfactory organs and a different mechanism of peripheral signal inception and processing, but the resemblances in neuroanatomical logic and physiological coding properties compare with those in mammals (Silbering and Benton, 2010). Also, there is a comparable function among members in their relative stages of olfactory signal transduction pathways. For instances, although odorant-binding proteins (OBPs) in mammals and insects are not homologous, they are both expressed in high concentration around olfactory dendrites, and play a critical role at the first step of olfactory signal transmission. OBPs and the structurally-related pheromone binding proteins (PBPs) are the first proteins to interact with the odor and, by an inherent binding preference determined by their ligand pocket that is formed by six α-helices (Pelosi, 2006), may help determine odor responses. Moreover, odor recognition is likely a coordinated process requiring the combined specificities contributed by OBPs and ORs and thus, optimal tuning and sensitivity of an olfactory sensillum would result when there is expression in the same sensillum of an OBP and an OR binding the same class of odor molecules (Grosse-Wilde, 2006, van der Goes *et al*., 2007). Thus, OBPs are potentially key components of receptor cell specificity as defined by levels of sensitivity to specific odorants.

Knowledge of parasite–mosquito interactions is essential to develop strategies that will reduce malaria transmission through the mosquito vector. Current research efforts are focused on the development of new drugs for the disease, development of vaccines against the parasite (Kar, 2010; Gershon, 2002), and mosquito vector transgenesis for the generation of mosquitoes refractive to parasite infection, multiplication and/or development (Kim, 2004; Ito, 2002). To date, however, the most successful approaches for control of malaria transmission have been based on methods that aimed at a reduction of the frequency of contact between the mosquito vectors and their human targets.

One of the approaches for controlling mosquito borne diseases is the interruption of disease transmission through mosquito control or avoiding mosquito bites. Plant products as potential insecticides or repellents can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at community level (Potter, 2005). Second to insecticides, which represent a serious burden for the environment and human health (Eskenazi, 2009; van den Berg, 2009), and whose prolonged use results in the selection of resistant vector populations (Ranson, 2009), mosquito repellents are the most commonly used agents for

prevention of infection by keeping infected mosquitoes away from human targets and preventing an infected human from spreading the parasite to uninfected mosquitoes (Moore, 2007).

In contrast to whole herbs, recent studies have confirmed that plant extracts form wide range of plants are more advantageous and efficacious. Among the plant families with promising essential oils used as repellents, *Cymbopogon*, *Ocimum* and *Eucalyptus* are the most cited (Venugopal and Gaddaguti, 2015). In traditional medicine, patchouli (*Pogostemon cablin*), cananga (*Canangium odoratum*), lemongrass (*Cymbopogon citratus*) and citronella (*Cymbopogon nardus*) are often used as insect repellents (TRECYDA, 2011). The main objective of the present investigation is to explore the possibility of repellent activity of potential natural compounds from *Ocimum* species, citronella (*Cymbopogon* species), catnip (*Nepeta cataria*), *Hierochloe odorata*, vertiver (*Vertver zizanioides*), *Eugenia aromatic*, *Artemisia monosperma* against odorant binding proteins of *Anopheles gambiae* and *Anopheles stephensi* (Manorenjitha *et al*., 2013; Chauhan and Raina, 2006; Soliman and El-Sherif, 1995; How, 2008; Ritchie, 2006; Hsu *et al*., 2013; Teketee, 2010; Hao, 2008; Hill, 2007). Numerous plants with insect repelling properties are native to the tropics where they are produced for a wide range of medicinal purposes. The prime objective of the present investigation is to explore the possibility of repellent activity of potential natural compounds from different plant species against odorant binding proteins of *Anopheles gambiae* and *Anopheles stephensi*. In this study, an attempt was made to understand possible mechanism that governs the interactions of natural mosquito repellent compounds against odorant binding proteins of *A. gambiae* and *A. stephensi*. Further these studies will widen the scope to choose the most suitable compounds for design and development of effective and safe mosquito repellents.

The present research proposal has been designed with the following objectives which are as follows:

OBJECTIVES

1. Screening of phytochemicals reported as repellant from published literature and public domain.
2. Identification and characterization of binding sites of target Odorant Binding proteins (OBPs) from *Anopheles* species.
3. *In silico* docking and screening of the compounds with higher binding energy as compared to widely used repellent DEET.

CHAPTER II

Review of Literature

Vector Borne Disease (VBD)

Vector-borne diseases (VBDs) are illnesses caused by pathogens and parasites in human populations. These vector-borne diseases are malaria, dengue, schistosomiasis, human African trypanosomiasis, Leishmaniasis, Chagas disease, yellow fever, *Japanese encephalitis* (JE) and onchocerciasis etc. Emerging and resurging vector-borne diseases cause significant morbidity and mortality, especially in the developing world (Gratz, 1999; Eisen, 2011; Mackey *et al*., 2014; Wu *et al*., 2016). Technological advances over the last decades with relevance to VBDs include the emergence of molecular techniques for vector species identification and pathogen detection and identification, and a rapid evolution in hardware and software options to support data collection, management, and analysis. These advances are now dramatically changing our capacity to predict, prevent, and control VBDs (Lars and Eisen, 2010).

Malaria

Vectors transmit pathogens and parasites from one infected person (or animal) to another, causing serious diseases in human populations. Malaria commonly found in tropical and sub-tropical regions and places where access to safe drinking-water and sanitation systems is problematic**.** Vector-borne diseases account for 17% of the estimated global burden of all infectious diseases. World-wide, an estimated number of 3.4 billion people are still at risk of malaria. In 2012 approximately 207 million cases of malaria occurred globally with most cases (80%) and deaths (90%) occurring in Africa. Most deaths (77%) occur in children under the age of five (WHO, 2013). Examples of some vector-borne diseases include Dengue fever, West Nile Virus, Lyme disease, and malaria.

Malaria is caused by five *Plasmodium* species, namely, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*. Among these *P. vivax* are the most prevalent, and *P. falciparum* is the most dangerous, with the highest rates of complications and mortality (Alam, 2014; Biamonte, 2012). Malaria is an acute febrile illness. In a non-immune individual, symptoms appear seven days or more (usually between 10 and 15 days) after the infective mosquito bite. The first symptoms – fever, headache, chills and vomiting – may be mild and difficult to recognize as malaria. If not treated within 24 hours, *P. falciparum* malaria can progress to severe illness, often leading to death. Children with severe malaria frequently develop one or more of the following symptoms:

severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria (Kleinschmidt, 2009).

There are more than 100 types of *Plasmodium* parasites, which can infect a variety of species. Scientists have identified five types that specifically infect humans, (WHO, 2015) they are:

- ***P. falciparum*** - located worldwide in tropical and suburban areas, but predominately in Africa. An estimated 1 million people are killed by this strain every year. The strain can multiply rapidly and can adhere to blood vessel walls in the brain, causing rapid onset of severe malaria including cerebral malaria.
- ***P. vivax*** - located in Latin America, Africa, and Asia, it is arguably the most widespread due to the high population of Asia. This strain has a dormant liver stage that can activate and invade the blood after months or years, causing many patients to relapse.
- ***P. ovale*** - located mainly in West Africa, it is biologically and morphologically very similar to *P. vivax*. However, unlike *P. vivax*, this strain can affect individuals who are negative with the Duffy blood group, which is the case for many residents of sub-Saharan Africa. This explains the greater prevalence of *P. ovale* (rather than *P. vivax*) in most of Africa.
- ***P. malariae*** - located worldwide and the only human malaria parasite to have a three-day cycle. If left untreated, *P. malariae* can cause a long-lasting, chronic infection that can last a lifetime and which may cause the nephrotic syndrome.
- ***P. knowlesi*** - located in Southeast Asia and associated with macaques (a type of monkey). This strain has a 24 hour cycle and can, therefore, multiply rapidly once a patient is infected, causing an uncomplicated case to become serious very quickly. Fatal cases of infection with this strain have been reported.

Mode of transmission of malaria

Vector-borne exposure occurs when an insect acquires a pathogen from one animal and transmits it to another. Diseases can be transmitted by vectors either mechanically or biologically. Mechanical transmission means that the disease agent does not replicate or develop in/on the vector; it is simply transported by the vector from one animal to another (flies). Biological transmission occurs when the vector uptakes the agent, usually through a blood meal from an infected animal, replicates and/or develops it, and then regurgitates the pathogen onto or injects it into a susceptible animal.

The female *Anopheles* mosquito is the vector for human malaria. Some 60 species of this mosquito have been identified as vectors for malaria, and their distribution varies from country to country. The infection is transmitted by the bite of an infected female. The mosquito usually bites during dawn and dust time. The mosquito becomes infected by biting a patient with malaria infection. When a mosquito bites an infected individual, it sucks the gametocytes, the sexual forms of the parasite, along with blood. These gametocytes continue the sexual phase of the cycle and the sporozoites fill the salivary glands of the infested mosquito. Once the mosquito becomes infected, it remains so far life. The female mosquitoes can survive up to 4 weeks under normal temperature *i.e*. 28°C to 30°C and humidity *i.e*. 60 to 80%. When this female mosquito bites the man for a blood meal, which it needs to nourish its eggs, it inoculates the sporozoites into human blood stream, thus spreading the infection. (www.arogya.com).

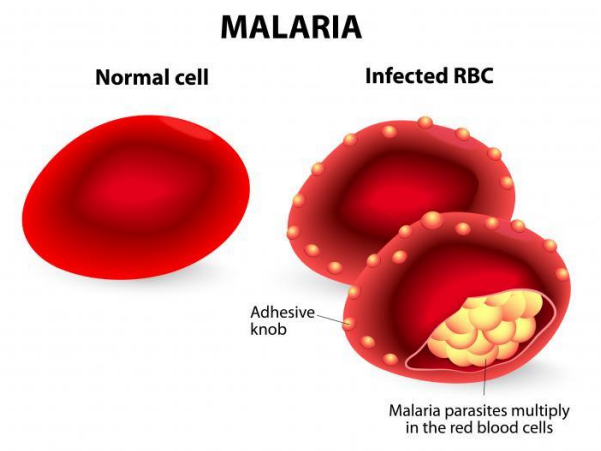

Figure 1: The multiplication of malaria parasites in RBC

Anopheles gambiae complex in Africa, *An. freeborni* in North America, *An. culicifacies, An. fluviatilis, An. minimus, An. philippinensis*, *An. stephensi*, and *An. sundaicus* in the Indian subcontinent (http://www.cdc.gov). *An. leucosphyrus*, *An. latens, An. cracens, An. hackeri, An. dirus* etc., have been identified as the vectors for the transmission of *P. knowlesi* (CDC malaria, Indra Vythilingam *et al.*, 2008).

Vectors of malaria

Vectors are living organisms that can transmit infectious diseases between humans or from animals to humans causing serious diseases in human populations. Many of these vectors are blood sucking insects, which ingest disease-producing microorganisms during a blood meal from an infected host (human or animal) and later inject it into a new host during their subsequent blood meal. Mosquitoes are the best known disease vector. Others include ticks, flies, sandflies, fleas, triatomine bugs and some freshwater aquatic snails (Lars Eisen, 2010; WHO, 2016).

Malaria is transmitted from man to man by the female anopheles mosquito, one of the most capable vectors of human disease. Various species have been found to be the vectors in different parts of the world. *A. gambiae* complex is the chief vector in Africa and *A. freeborni* in North America. Nearly 45 species of the mosquito have been found in India and *A. culicifacies, A. fluviatilis, A. minimus, A. philippinensis, A. stephensi, A. sundaicus*, and *A. leucosphyrus* have been implicated in the transmission of malaria. The areas of distribution are different for these mosquitoes: *A. fluviatilis, A. minimus* are found in the foot-hill regions, *A. stephensi, A. sundaicus* are found in the coastal regions, *A. culicifacies* and *A. philippinensis* are found in the plains. Species like *A. stephensi* are highly adaptable and are found to be very potent vectors of human malaria (http://www.malariasite.com/anopheles-mosquito/).

The female mosquito lays 30-150 eggs every 2-3 days. Human blood is needed to nourish these eggs and *Anopheles* shows the most regular cycles of blood feeding and egg laying. As a corollary, by using personal protective measures against mosquito bites, like using mosquito nets, one can deny the blood meal and hence help in mosquito control. *Anopheles* mosquitoes enter the house between 5 p.m. and 9.30 p.m. and again in early hours of morning. They start biting by late evening and the peak of biting activity is at midnight and early hours of morning. By keeping the windows and doors closed between 5 p.m. and 10 p.m. and again in early morning, one can prevent the entry of these mosquitoes into the house. Also protect yourself against the bites in the evenings and early mornings by wearing garments that cover the body as much as possible and at bedtime, by using mosquito nets without fail. The average life span of a mosquito is 2-3 weeks. It can be longer in ideal living conditions (http://www.malariasite.com/anopheles-mosquito/).

(A) (B)

Figure 2: Important vectors of malarial parasite i.e., *Anopheles gambiae* (A) and *Anopheles stephensi* (B)

2.4 Geographic Distribution *A. gambiae* and *A. stephensi*

Anopheles are found worldwide except Antarctica (as shown in fig. 2.3). Malaria is transmitted by different *Anopheles* species, depending on the region and the environment. *Anopheles* that can transmit malaria are found not only in malaria-endemic areas, but also in areas where malaria has been eliminated. The latter areas are thus constantly at risk of re-introduction of the disease (www.cdc.gov/malaria).

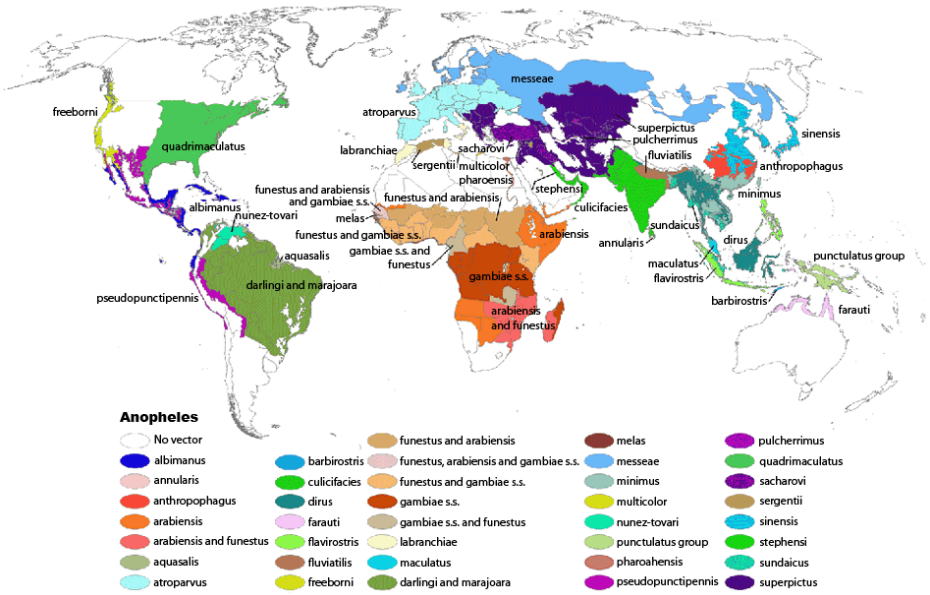

Figure 3: The map of the world showing the distribution of predominant malaria vectors

Current status of vector borne disease (malaria)

The month of June is observed as anti-malaria month (AMM) every year. The objectives of the AMM campaign is to bring about sustainable changes in the community behavior towards the elimination of malaria. As we are aware, Govt. of India has targeted for a malaria free India by 2030. The national frame work for malaria elimination in India (2016-2030) was lunched by Hon'ble Union Health Minister, Ministry of Health and Family Welfare, Government of India in February 2016 (http://www.nvbdcp.gov.in).

Vector Borne Disease Control Strategies

Vector Control

Mosquito control is an important component of malaria control strategy, although elimination of malaria in an area does not require the elimination of all *Anopheles* mosquitoes. Intervention measures to restrict the transmission of malaria by controlling the vector population form the main part of the vector control.

(i) **Chemical Control**

- Use of Indoor Residual Spray (IRS) with insecticides recommended under the programmer,
- Use of chemical larvicides like Abate in potable water,
- Aerosol space spray during day time,
- Malathion fogging during outbreaks.

(ii) **Biological Control**

- Use of larvivorous fish in ornamental tanks, fountains etc,
- Use of biocides.

(iii) Personal Prophylactic Measures that individuals/communities can take up

- Use of mosquito repellent creams, liquids, coils, mats etc.
- Screening of the houses with wire mesh,
- Use of bed nets treated with insecticide,
- Wearing clothes that cover maximum surface area of the body.

(iv) Environmental Management & Source Reduction Methods

- Source reduction i.e. filling of the breeding places,
- Proper covering of stored water,
- Channelization of breeding source. (http://www.nvbdcp.gov.in/)

Insect/Mosquito repellant

A mosquito repellent doesn't actually kill mosquitoes. Repellents work by making people less attractive to mosquitoes, so they're less likely to bite you. Female mosquitoes bite human beings every 3 to 4 days for the blood meal and use visual, thermal, and most importantly, olfactory stimuli to locate a host. Carbon dioxide, released mainly from breath but also from skin, serves as a long-range airborne attractant and can be detected by mosquitoes at distances of up to 36 meters. Lactic acid, skin temperature, moisture, other volatile compounds, derived from sebum, eccrine and apocrine sweat, or the cutaneous microflora bacterial action on these secretions, may all act as attractants. In addition, floral fragrances from perfumes, soaps, lotions, and hair-care products may also attract mosquitoes and consumption of alcoholic drinks such as beer can also increase the attractiveness to mosquitoes. These attractants stimulate the chemoreceptors on the antennae of the mosquitoes and inhibition of these receptors by certain chemicals can produce mosquito repellent effect. Several synthetic and natural substances are being used as mosquito repellents. DEET (N,N-diethyl-m-toluamide [or N,N-diethyl-3-methylbenzamide]), picaridin (1-methyl-propyl 2-[2-hydroxyethyl]-1-piperidinecarboxylate [also known as KBR 3023]), PMD (p-menthane 3,8-diole [or oil of

lemon eucalyptus]), MGK-326(dipropyl isocinchomeronate), MGK-264 (N-octyl bicycloheptane dicarboximide), IR3535 (ethyl butyl-acetylaminopropionate), and oil of citronella have been registered as insect repellents by the US Environmental Protection Agency (www.malariasite.com/personal-protection).

DEET

Disruption of the normal olfactory responses to odor molecules that control mosquito behavior provides a means to reduce human-mosquito interactions; a primary method for achieving this is the use of repellents. N, N-Diethyl-3-methylbenzamide (DEET) is considered the most broad spectrum and effective insect repellent available (Katz, 2008). Although effective, DEET is also known to be toxic (Briassoulis, 2001), to have reduced efficacy with time after application, and to be ineffective against species that develop resistance (Rutledge, 1978). DEET acting on OBPs to disrupt the interactions that they make with other OBPs or other components of the olfactory system could disrupt downstream activation of odorant receptors (Emma and Murphy, 2012). Later studies have provided evidence that DEET targets the function of insect odorant receptors (ORs) and, in the case of mosquitoes, blocks an OR/co-receptor complex which is involved in the recognition of 1-octen-3-ol, a component of human sweat (Ditzen, 2008). However, another recent study proposes that mosquitoes have the ability to smell and avoid DEET through a specific DEET-sensitive olfactory receptor neuron (ORN), housed in a trichoid sensillum, without inhibiting the reception of other chemical signals such as CO_2, lactic acid or 1-octen-3-ol (Syed, 2008).

Traditional method used for mosquito repellent

This repellency of plant material has been exploited for thousands of years by man, most simply by hanging bruised plants in houses, a practice that is still in wide use throughout the developing countries (Moore SJ, 2006). Plants have also been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance mosquitoes and later as oil formulations applied to the skin or clothes which was first recorded in writings by ancient (Greek, 1996; Roman, Owen T, 1805 and Indian scholars Johnson T, 1998). Traditionally, various types of substances have been used to repel mosquitos. These include such things as smoke, plant extracts, oils, tars, and muds. The tribal people utilized mainly a combination of the dried stem and leaf of the plant, seed oil which is burnt to elicit its repellent activity against hematophagous insects. The dry leaves and leaf extract of Herbal plants like *Homalium nepalense, Lantana camara,., Ocimum sanctum, Vitex nigundo, Ageratum conyzoides, Tinospora cordifolia, Ocimum canum, Ocimum gratissimum, Barleria prionitis., Clerodendrum viscosum, Clerodendrum induicum., Justicia adhatoda., Annona squamosa.,*

Woodfordia fruticosa, *Swertia angustifolia* and whole part of these plants like *Michelia champaca*, *Clerodendrum viscosum* and *Andrographis paniculata* have found extensive use as mosquito repellent (Pattanayak, 2015). Essential oils and extracts belonging to plants in the citronella genus (Poaceae) are commonly used as ingredients of plant-based mosquito repellents. In traditional medicine, patchouli (*Pogostemon cablin*), cananga (*Canangium odoratum*), lemongrass (*Cymbopogon citratus*) and citronella (*Cymbopogon nardus*) are often used as insect repellents (TRECYDA, 2011; Solomon, 2012). Plant essential oils such as citronella have become popular for use as safe mosquito repellents (Fradin, 1998; Bhupen and Kalita, 2013). Besides citronella oil, numerous other plant extracts have been available as insect repellents for protection against mosquitoes.

Phytochemicals/Plant based product as Repellant

There are many native plant species that have a history of use for personal protection against biting insects. Numerous plants with insect repelling properties are native to the tropics where they are produced for a wide range of medicinal purposes. Essential oils derived from a variety of plant species have shown considerable efficacy as repellents against various hematophagous arthropods, especially mosquitoes. The most effective plant species that have been used against mosquitoes include catnip (Lamiaceae), hairy basil (Lamiaceae), citronella (Poaceae), vetiver (Poaceae) and clove (Myrtaceae) (Polsomboon *et al.*, 2008, Suwansirisilp *et al.*, 2012, Sathantriphop *et al*., 2014). These five plants produced comparative yields of essential oils. The yield from catnip (4.60-5.70 %) was higher than other plant species followed by vetiver (2.10-2.90 %), clove (1.28 %), citronella (0.68%) and hairy basil oil (0.05 %) respectively (Chungsamarnyart and Jiwajinda, 1992; Dethier *et al*., 1997; Louey *et al*., 2001; Kerdchoechuen *et al*., 2010). Plants of the genus *Cymbopogon* are already commercially cropped in malaria endemic countries including South America, especially Brazil (6 million trees), southern China, India, Sri Lanka, Congo (Zaire), Kenya and most countries in southern Africa, where it is grown for essential oil production and timber (*Corymbia citriodora*). Local production of insect repellent would remove the high cost of importation in developing countries. The list of compounds which are reported to be used as mosquito repellent from various literature have been summarized in **Table 1**.

Table 1 List of compounds derived from plant used as repellants

Name	**Location**	**Repellent compounds**	**plant extract**	**References**
Eucalyptus spp.	Ethiopia	1,8-cineolecitronellal	Leaves	Ansari,2005
Eugenia aromaticu	India	Eugenol, cinnamoldehyde, carvacrol, thymol	Seed oil	CampbellC,2010
Lantana javanica	Kenya	Camphor	Plant extract	Hao H,2008
Ocimum americanum (Hairy basil)	Nigeria	Linoleic acid, Methyl cinnamate, d-camphor, E-citral, Zcitral, eugenol, methylchavicol, terpneol, farnesene, myrcene, alpha-pinene, beta-pinene, 1,8-cineol, camphene, beta-bisabolene, , alpha-terpinene, limonene, linalool, linalyl acetate, methyl-eugenol, iso-eugenol, farnesol, Ocimene, terpinolene, geraniol, beta-caryophyelene, germacrene D, geranyl iso-butyrate, alpha-cadinol (these are chemical composition of hairy basil oil)	Leaves and seeds	Hill N,2007, TCFF; Khalid, 2006
Mentha arvensis	Malaysia	Myrcene	Leaves	Syed Z,2008

Cymbopogon nardus (citronella)	Brazil	Citronella, Citral, citronellal, limonene, myrcene, geraniol, trans-citral, ciscitral, geranyl acetate, citronellol, gamma-terpineol, cis-sabinene hydrate, linalool, beta-caryophyllene (these are chemical composition of citronella oil)	Seed oil	Fradin MS, 2007; Hsu et al., 2013; Nakahara et al., 2003
Azadirachta indica (neem)	Ethiopia,India	Azadirachtin , saponins	Leaves and plant extract	Ritchie SA,2006
Artemisia monosperma	Brazil	limonene	Leave extract and bark	How H,2008
Zanthoxylum limonella	Thailand		Seed oil	Caroll SP,2006
Curcuma longa	Nigeria		Seed oil	Hill N,2007
Hierochloe odorata		phytol , coumarin	leaves	
Ocimum tenuiflorum var. *cimayu*		2-Hexadecen-1-ol, Phytol, Lycopersin, Phenol-2-Methoxy-3-(2-Propenyl), DL-alpha-Tocopherol, Gamma-Sitosterol, Benzene, 1,2-Dimethoxy-4-(2-Propenyl)	leaves	Manorenjitha et al. 2013
O. basilicum var. *pilosum* (willd)-Benth		4H-1-Benzopyran-4-One, 5-Hydroxy-6,5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl),catechol,phytol,2-	leaves	Manorenjitha et al. 2013

		hydroxy-6-methylbenzaldehyde,Monoacetin		
Nepeta cataria (Catnip)		Z,E nepetalactone, E,Z-nepetalactone, Z,E-nepetalic acid, E,Znepetalic acid		Chauhan and Raina, 2006
Syzygium aromaticum (clove)		Eugenol, β-caryophyllene		Park and Shin, 2005; Prashar et al., 2006
Vetiveria zizanioides (Vetiver)		terpinen-4-ol, 5-epiprezizane, khusimene, alpha-muurolene, khusimone, calacorene, beta-humulene, alpha-longipinene, gamma-selinene, -selinene, -cadinene, valencene, calarene,-gurjunene, alpha-amorphene, epizizanal, 3-epizizanol, khusimol, Iso-khusimol, Valerenol, alpha-vetivone, beta-vetivone (these are chemical composition of vertiver oil)		Soliman and El-Sherif-1995

Molecular targets of plant based compounds

Several studies have shown that the natural mosquito repellent compounds possible interact with odorant binding proteins (OBPs) of malarial vectors.

Odorant Binding Proteins (OBPs)

The olfactory system of mosquitoes plays a crucial role in their survival and reproductive success as it facilitates sugar feeding (Foster, 1994), mating (Cabrera, 2007), and oviposition (Bentley, 1989) as well as host detection and blood feeding (Takken, 1991). Odorant-binding proteins (OBPs) are the first components of their odor detection unit. Hydrophobic odorant molecules entering the aqueous sensillar lymph of insect antennae are captured by OBPs that solubilize, carry, and deliver them to their cognate odorant receptors (Leal, 2003). The fact that OBPs are so far the best characterized olfactory macromolecules has identified them as potential targets for the design of novel insect repellents or attractants. Till date, 69 odorant binding proteins have been reported in *A. gambiae* and typically all OBPs may not be involved in recognition of host for their blood meal (Venugopal and Gaddaguti, 2015). The arthropod OBPs form a large specific multi-gene family. They are 10–30 kDa globular and water-soluble proteins that are characterized by a specific six a-helical domain comprising of six highly conserved cysteines that have distinct disulphide connectivities. These structural features are now considered the hallmark of this protein family (Calvo *et al.*, 2002; Valenzuela *et al*., 2002; Calvo *et al*., 2006). OBPs have been identified in a number of insect species in *A. gambiae* (Vogt, 2002; Xu *et al*., 2003; Zhou *et al*., 2004; Li *et al*., 2005; Vieira and Rozas, 2011).

Sub-families of OBP genes in mosquitoes

In mosquitoes, three subfamilies of OBP genes have been characterized so far:

(i) The Classic OBPs that carry the six conserved cysteines characteristic motif of the OBP family.

(ii) The PlusC OBPs that have the same conserved cysteines and disulphide connectivity but which contain six additional cysteines with novel disulphide connectivities.

(iii) The Atypical OBPs that are among the longest known OBPs and that have initially been described as containing a single Classic OBP domain in its N-terminal extended by a less characterized C-terminal extension. Very recently, it was shown that Atypical OBPs comprises two domains that are in fact homologous to the Classic OBP domain and were hence considered as “dimer OBPs” (Vieira and Rozas, 2011).

Table 2: The different classes of OBPs in *Anopheles gambiae*

OBP	Subfamily			Not Determined	total	Reference
	Classic	PlusC	Atypical			
Previously Reported	29	16	16	-	61	Vogt, (2002); Xu et al., (2003); Zhou et al., (2004); and Vieira and Rozas, (2011)
Newly Identified	-	4	-	4	8	Malini and Manoharan, (2012)
Total identified till date - 69						

Computational approaches towards effective design of repellant

In recent study by conducted Venugopal and co-workers (2015) have revealed that 12 phytochemical compounds from *Ocimum* species targets *A. gambiae* odorant binding proteins (PDB ID: 3N7H and 3Q8I) through a computational analysis. OBP orthologs have been identified using the reciprocal BLAST hit approach (Moreno-Hagelsieb and Latimer, 2008) which is widely used in the detection of orthologs. Malini Manoharan *et al*. (2012) characterized genomic distribution and orthologous and phylogenetic relationships and give a comprehensive comparative genomics study of odorant binding proteins (OBPs) in the three disease-transmitting mosquito species *Anopheles gambiae, Aedes aegypti,* and *Culex quinquefasciatus* starting with the identification of 110 new OBPs in these three genomes. Tsitsanou *et al*., (2012) have characterized the high resolution crystal structure of an OBP of *Anopheles gambiae*, in complex with N,N-diethyl-m-toluamide (DEET), they modeled the interactions for this protein with 29 promising leads reported in the literature to have significant repellent activities, and carried out fluorescence binding studies with four highly ranked ligands. Venugopal and Gaddaguti *et al*., (2012) have identified 13 compounds from aerial parts of *Hyptis suaveolens* L. methanolic extracts. Further they have performed molecular docking studies of 13 compounds along with commercially known mosquito repellent compounds including DEET, Prallathrin, and Permithrin against Odorant Binding Protein (PDB ID: 3N7H) of *Anopheles gambiae* for further possibility for designing of potential mosquito repellent natural compounds. With the availability of high resolution crystal structures of OBPs from malarial vectors and reported plant based compounds as repellent will help in designing effective and non-toxic repellents in near future.

CHAPTER III

Materials and methods

Literature survey on phytochemicals as repellent

Plant-based repellents have been used for generations in traditional practice as a personal protection measure against host-seeking mosquitoes. Knowledge on traditional repellent plants obtained through ethnobotanical studies is a valuable resource for the development of new natural repellents. So, an extensive literature survey was conducted to retrieve articles on phytochemicals as repellent from public domains through key-word search and phrase search. The important phytochemicals with repellent activity from 10 different plants *i.e. Cymbopogon nardus, Artemisia monosperma, Hierochloe odorata, Ocimum americanum, Ocimum tenuliflorum, Nepeta cataria, Eugenia aromatic, Ocimum basilicum, Vertiveria zizanioides* etc. were screened from previously published literatures.

Selection of phytochemical compounds

A total of 40 compounds were finally selected as potent repellent and the coordinates (2D structure) were obtained from PubChem Compound database (http://pubchem.ncbi.nlm.nih.gov/). The 2D coordinates of these compounds were converted to 3D using Discovery Studio Visualizer 4.0 (DSV).

Target protein selection

3-dimensional modeling of target OBPs

Insect odarant binding proteins (OBPs) are components of olfactory system that binds to attractant and repellent odors. Odorant binding proteins are thought to be the primary proteins involved in the transport of odorants and pheromones to the olfactory receptor. Henceforth, they are considered as important targets for development of new repellents. Several crystal structure of OBPs from various mosquitoes and insects have been reported in PDB. But, no crystal structures of Odorant Binding Proteins (OBPs) of *Anopheles stephensi* is reported at RCSB (PDB). So, an attempt was made to elucidate the 3-dimensional structure of these proteins using available computational tools. The full length primary sequence of *Anopheles stephensi* OBPs *(i.e.,* UniProtKB Accession: D2IGZ8 and B5A5T7) were downloaded from UniProtKB. Comparative modeling is considered as one of the most accurate computational methods to

generate reliable theoretical 3-D model of protein from its primary amino acid sequence. But in this case, BLASTP and DELTA-BLAST search could not identify a suitable template for homology modeling of these OBPs from *Anopheles stephensi*. Therefore, fold-recognition methods/threading approach implemented in I-TASSER and LOMETs was used to generate the 3-dimensinal structure of these two OBPs. The models with lowest dope score from these tools was selected as best model for further refinement using WHAT-If server.

Crystal structure of OBPs from *Anopheles gambiae*

As the several crystal structures of odorant binding proteins (OBPs) from *Anopheles gambiae* were reported in PDB, a filter was applied retrieve seven crystal structures (below <2.5 Å resolution). A total of seven crystal structure OBPs i.e., OBP (PDB ID: 3PM2), OBP1 (PDB ID: 2ERB), OBP4 (PDB ID: 3Q8I), OBP7 (PDB ID: 3R1P), OBP20 (PDB ID: 3V2L), OBP22a (PDB ID: 3L4A) and OBP48 (PDB ID: 4IJ7) were downloaded and subjected to protein preparation using DSV. Prior to docking the OBPs were prepared by removal of heteroatoms including substrates and small molecules along with waters of crystallization. The active site residues were noted/identified from published literature for docking studies.

Molecular docking of compounds to the active site of OBPs

In order to explore the binding mechanism of phytochemicals with the target proteins, molecular docking studies have been performed using AutoDock 4.2.6 (ref) and AutoDock Tools 1.5.6. Initially before docking simulations, hydrogen atoms were added to the polar atoms and the Kollman charges were assigned for all atoms of the target order binding proteins. All rotating bonds, torsional degrees of freedom, atomic partial charges and non-polar hydrogens of the ligands were assigned. The docking simulations were conducted with different grid dimension covering all the putative residues involved in compound recognition obtained from published literature. For each docking simulation, a grid spacing of 0.375 Å was used under the hybrid Lamarckian Genetic Algorithm with the number of docking runs was set as 30. The Lamarckian genetic algorithm (LGA), considered one of the best docking methods available in Autodock, was adopted to perform the molecular docking studies. The parameters for LGA were defined as follows: a maximum number of 250,000 energy evaluations, a maximum number of generations of 27,000, and mutation and crossover rates of 0.02 and 0.8, respectively. Final docked conformations were clustered using a tolerance of 2Å RMSD. The docked conformations of each

ligand were ranked into clusters based on the binding energy and the top ranked conformations were visually analyzed.

Docking DEET in to the active site of OBPs

In this study the most widely used repellent i.e, DEET was docked in to the active site of OBPs with same grid and docking parameter used for docking of phytochemicals (mentioned above). The results of DEET was considered as reference to gauge the accuracy the docking and estimate the binding affinity of phyto-chemicals to OBPs of *A. stephensi* and *A. gambiae*.

Analysis of docking results

The non-bonded interaction including hydrogen bonding, hydrophobic and electrostatic interactions between docked compounds and target OBPs were analyzed using PyMol, DS and LigPlot+ v.1.4. All the artwork was created using Pymol, Photoshop CS5 and Illustrator CS6. A detailed analysis of residues involved in the interaction between ligands and protein was conducted using the Discovery Studio 4.0 Visualizer software (Accelyrs Software Inc., Discovery Studio Modeling Environment, and San Diego: Accelrys Software Inc., 2012).

CHAPTER IV

Results and discussion

Literature survey on plant based compounds as mosquito repellent

Initially around more than 100 published literatures (mostly from ethnobotanical/ethno pharmacological surveys) on plant based compounds as repellent were collected from public domains. All total, a set of 40 phytochemical compounds were screened from 10 common plants used as mosquito repellant (as summarized in **Table 3**).

Table 3

List of the plants along with the details of plants parts used as mosquito repellent obtained from primary literature survey

Sl. No.	**Name of the plants**	**Common name**	**Location**	**Part used as repellant**
1	*Eugenia aromaticu*		India	Seed oil
2	*Ocimum americanum*	Hairy basil	India	Leaves and seeds
3	*Cymbopogon nardus*	citronella	India	Seed oil
4	*Azadirachta indica*	neem	Ethiopia, India	Leaves and plant extract
5	*Artemisia monosperma*		Arabian dessert, Egypt	Leave extract and bark
6	*Hierochloe odorata*	Sweet grass	Europe, Asia, North America	leaves
7	*Ocimum tenuiflorum*	Holy basil/ tulasi	South Asia	leaves
8	*Ocimum basilicum*	Sweet basil	Asia	leaves
9	*Nepeta cataria*	Catnip	Asia, Europe, China	leaves
10	*Vetiveria zizanioides*	Vetiver	India	root

Mining of phytochemical compounds

The 2-dimenisonal structure of the screened phytochemical compounds reported as repellant from public literature were downloaded from PubChem database. The 2D structures were converted to 3D format using DSV4.0. The list of 40 compounds from different plants along with their molecular formula has been displayed in **Table 4**.

Table 4

List of the phytochemical compounds along with their compound ID and molecular formula obtained from PubChem database.

Name of the compound	**Source**	**Compound ID (CID)**	**Molecular Formula**
Citronellal	*Cymbopogon nardus*	7794	$C_{10}H_{18}O$
Citral	*Cymbopogon nardus*	638011	$C_{10}H_{16}O$
Myrcene	*Cymbopogon nardus*	31253	$C_{10}H_{16}$
Geraniol	*Cymbopogon nardus*	637566	$C_{10}H_{18}O$
Linalool	*Cymbopogon nardus*	6549	$C_{10}H_{18}O$
Azadirachtin	*Azadirachta indica*	5281303	$C_{35}H_{44}O_{16}$
Saponin	*Azadirachta indica*	198016	$C_{58}H_{94}O_2$
Limonene	*Artemisia monosperma, Cymbopogon nardus*	22311	$C_{10}H_{16}$
Phytol	*Hierochloe odorata, Ocimum tenuiflorum*	5280435	$C_{20}H_{40}O$
Coumarin	*Hierochloe odorata*	323	$C_9H_6O_2$
Linoleic Acid	*Ocimum americanum*	5280450	$C_{18}H_{32}O_2$
Methyl Cinnamate	*Ocimum americanum*	637520	$C_{10}H_{10}O_2$
Camphor	*Ocimum americanum*	2537	$C_{10}H_{16}O$
Ocimene	*Ocimum americanum*	5281553	$C_{10}H_{16}$
Alpha-Pinene	*Ocimum americanum*	6654	$C_{10}H_{16}$
Beta-Pinene	*Ocimum americanum*	14896	$C_{10}H_{16}$
Citral	*Ocimum americanum*	638011	$C_{10}H_{16}O$
Eugenol	*Ocimum americanum*	3314	$C_{10}H_{12}O_2$

Lycopersin	*Ocimum tenuiflorum*	5281730	$C_{20}H_{14}O_8$
Phenol-2-Methoxy-3-(2-Propenyl)	*Ocimum tenuiflorum*	596373	$C_{10}H_{12}O_2$
Alpha-Tocopherol	*Ocimum tenuiflorum*	2116	$C_{29}H_{50}O_2$
Gamma-Sitosterol	*Ocimum tenuiflorum*	457801	$C_{29}H_{50}O$
Nepetalactone	*Nepeta cataria*	92770	$C_{10}H_{14}O_2$
Nepetalic Acid	*Nepeta cataria*	5486616	$C_{10}H_{16}O_3$
4h-1-Benzopyran-4-one	*Ocimum basilicum*	10286	$C_9H_6O_2$
2-Hydroxy-6-methylbenzaldehyde	*Ocimum basilicum*	585174	$C_8H_8O_2$
Catechol	*Ocimum basilicum*	289	$C_6H_6O_2$
Monoacetin	*Ocimum basilicum*	33510	$C_5H_{10}O_4$
Carvacrol	*Eugenia aromaticu*	10364	$C_{10}H_{14}O$
Alpha-Muurolene	*Vetiveria zizanioides*	12306047	$C_{15}H_{24}$
Alpha-Cadinene	*Vetiveria zizanioides*	12306048	$C_{15}H_{24}$
Khusimone	*Vetiveria zizanioides*	6428327	$C_{14}H_{20}O$
Calacorene	*Vetiveria zizanioides*	5315609	$C_{15}H_{20}$
Beta-Humulene	*Vetiveria zizanioides*	21159064	$C_{15}H_{24}$
Alpha-Longipinene	*Vetiveria zizanioides*	12311396	$C_{15}H_{24}$
Gamma-Selinene	*Vetiveria zizanioides*	521334	$C_{15}H_{24}$
Calarene	*Vetiveria zizanioides*	15560278	$C_{15}H_{24}$
Gurjunene	*Vetiveria zizanioides*	15560275	$C_{15}H_{24}$
Alpha-Vetivone	*Vetiveria zizanioides*	442405	$C_{15}H_{22}O$
Beta-Vetivone	*Vetiveria zizanioides*	442406	$C_{15}H_{22}O$
Khusimol	*Vetiveria zizanioides*	167519	$C_{15}H_{24}O$

Target odorant binding protein (OBPs) from *Anopheles gambiae*

A total of seven crystal structures (below <2.5Å resolution) of OBPs from ***Anopheles gambiae*** *i.e.* OBP (PDB ID: 3PM2), OBP1 (PDB ID: 2ERB), OBP4 (PDB ID: 3Q8I), OBP7 (PDB ID: 3R1P), OBP20 (PDB ID: 3V2L), OBP22a (PDB ID: 3L4A), OBP48 (PDB ID: 4IJ7) were downloaded and further subjected to protein preparation using Discovery studio visualizer (as shown in **Table 5**). Prior to docking the OBPs were prepared by removal of heteroatoms including substrates and small molecules along with waters of crystallization. The active site residues were noted/identified from published literature and the crystal structure for docking studies.

Threading of target protein

For odorant Binding Proteins (OBPs)of *Anopheles stephensi* fold recognition method (threading) implemented in I-TASSER, LOMETS, and RAPTROX were used to obtain a 3-dimensional model of these OBPs (i.e., UniProtKB ID: D2IGZ8; OBP1 and B5A5T7; OBP7) (**Table 5**). The models with lowest dope score from these threading servers were selected as best model for further optimization and refinement.

Table 5

List of the Odorant Binding Proteins (OBPs) of *Anopheles gambiae* and *Anopheles stephensi*

NAME	SOURCE	UNIPROTKB ID	PDB ID/Threading	NO. OF AMINO ACID	MOL. WT. (kDa)	pI (isoelectric point)
obp1	*Anopheles stephensi*	D2IGZ8	Modelled	144	16.564	5.67
obp	*Anopheles gambiae*	Q7PF80	3PM2(58-228)	228	24.756	8.19
obp1	*Anopheles gambiae*	Q8I8T0	2ERB(20-144)	144	16.553	5.53
obp4	*Anopheles gambiae*	Q8T6R7	3Q8I(27-150)	150	16.702	6.8
obp7	*Anopheles stephensi*	B5A5T7	Modelled	146	16.38	5.27
obp7	*Anopheles gambiae*	Q7PXT9	3R1P(29-154)	154	17.437	5.3
obp20	*Anopheles gambiae*	Q7Q9J3	3V2L(24-142)	142	16.061	9.18
obp22a	*Anopheles gambiae*	Q7PGA3	3L4A(22-144)	144	16.174	4.18
obp48	*Anopheles gambiae*	Q8MMI9	4IJ7(29-200)	200	22.006	5.14

Docking results of phytochemicals in to the active sites of OBPs

Total 40 no. phytochemical compounds were docked against the 7 OBPs of *Anopheles gambiae* and 2 modelled structure of *Anopheles stephensi.* A total of the 40 compounds tested for their binding affinity with odorant binding proteins of *Anopheles gmabiae*, where azardirachtin (*Azadirachta indica*), Lycopersin (*Ocimum tenuiflorum*), khusimol (*Vetiveria zizanioides*) exhibit strong binding affinity with OBP (3PM2). Azardirachtin (*Azadirachta indica*), alpha-tocopherol, gamma-sitosterol, lycopersin, Phenol-2-Methoxy-3-(2-Propenyl) of *Ocimum tenuiflorum*, alpha-vetivone, beta-vetivone, gamma-selenene, khusimol, khusimone of *Vetiveria zizanioides* showing strong binding affinity with OBP1 (PDB ID: 2ERB). Alpha-tocopherol, gamma-sitosterol, lycopersin, of *Ocimum tenuiflorum,* azardirachtin, saponin of *Azadirachta indica* and alpha-vetivone of *Vetiveria zizanioides* showing strong binding affinity with OBP4 (PDB ID: 3Q8I). alpha-tocopherol, gamma-sitosterol, gurjunene, lycopersin of *Ocimum tenuiflorum,* azardirachtin, saponin of *Azadirachta indica,* alpha-vetivone, alpha-longipipene, alpha-murolene, alpha-cardinene, beta-vetivone, calarene, gamma-selenene, khusimol, khusimone of *Vetiveria zizanioides* showing strong binding affinity with OBP7 (PDB ID:3R1P). Phenol-2-Methoxy-3-(2-Propenyl), lycopersin, gamma-sitosterol of *Ocimum tenuiflorum,* khusimone, khusimol, gamma-selenene, alpha-vetivone, beta-vetivone, beta-humulene, alpha-longipipene of *Vetiveria zizanioides* showing strong binding affinity with OBP20 (PDB ID: 3V2L). alpha-tocopherol gamma-sitosterol, gurjunene of *Ocimum tenuiflorum,* azardirachtin, saponin of *Azadirachta indica,* alpha-longipipene, alpha-cardinene, alpha-murolene, alpha-vetivone, beta-humulene, beta-vetivone , calacorene, calarene, gamma-selenene, khusimol, khusimone of *Vetiveria zizanioides* showing strong binding affinity with OBP22a (PDB ID: 3L4A). Azardirachtin of *Azadirachta indica and* gamma-sitosterol, lycopersin of *Ocimum tenuiflorum* showing strong binding affinity with OBP48 (PDB ID: 4IJ7).

Gamma-Sitosterol, Phenol-2-Methoxy-3-(2-Propenyl) of *Ocimum tenuiflorum* Azardirachtin, saponin of *Azadirachta indica,* alpha-cardinene, alpha-longipipene, alpha-vetivone, beta-vetivone, calarene, gamma-selenene, khusimol, khusimone of *Vetiveria zizanioides* showing strong binding affinity with OBP1 of *Anopheles stephensi*. Lycopersin and Phenol-2-Methoxy-3-(2-Propenyl) of *Ocimum tenuiflorum* showing strong binding affinity with OBP7 of *Anopheles stephensi*. In this study we have used the widely used repellent DEET as reference to the estimate he binding affinity of the plant based repellents (**Table 6)**.

Table 6 Docking score of potential phytochemical compound

Target protein	**Source of target protein**	**PDB ID/ Modelled**	**Name of the compound (Ligand)**	**Binding Energy (KCal/mol)**	**No. of H Bonds**	**Average distance of H-bonds**	**Residues forming H-bonds**	**Binding energy with DEET (reference)**
OBP1	*Anopheles stephensi*	(I-Tasser)	Gamma-Sitosterol	-8.05	1	1.84079	LEU77	-6.55
			Phenol-2-Methoxy-3-(2-Propenyl)	-10.04	1	2.19825	PHE142	-
			azardirachtin	**-13.77**	**7**	**2.5623**	**PHE142, VAL144, ALA107, LEU143, HIS140,**	-
			alpha-cardinene	-8.13	-	-	-	-
			alpha-longipipene	-8.17	-	-	-	-
			alpha-vetivone	-8.28	2	2.334	VAL144, LEU143	-
			beta-vetivone	-8.41	1	2.91031	GLY111	-
			calarene	-8.01	-	-	-	-
			gamma-selenene	-8.3	-	-	-	-
			khusimol	**-8.05**	**2**	**2.96308**	**VAL144 , PHE142**	-
			khusimone	-8.44	1	2.81703	GLY111	-
			saponin	-11.01	13	3.0005	GLY111, VAL144, MET38, ALA107, HIS96, HIS130, ALA98, TRP133	-
OBP	*Anopheles gambiae*	3PM2	**azardirachtin**	**-10.05**	**3**	**3.236903**	**CYS54, THR21, PHE172**	-6.29

			lycopersin	**-9.79**	**7**	**3.1002**	**PHE127, ALA139, GLY142,GLY135**	-
			khusimol	**-8.06**	**2**	**2.5364**	**LEU24, VAL25**	
OBP1	*Anopheles gambiae*	2ERB	**azardirachtin**	**-12.33**	**3**	**3.155663**	**LEU124, VAL125, SER79**	-6.22
			alpha-tocopherol	-8.55		-	-	-
			gamma-sitosterol	-9.96	1	1.73265	ALA88	-
			lycopersin	**-10.42**	**8**	**3.188396**	**VAL125, PHE123, HIS111, TYR122, TYR10, HIS121, PHE123**	
			Phenol-2-Methoxy-3-(2-Propenyl)	-8.21	-	-	-	-
			alpha-vetivone	-8.33	1	2.70769	VAL125	-
			beta-vetivone	-8.35	1	3.12051	VAL125	-
			gamma-selenene	-8.02	-	-	-	-
			khusimol	**-8.16**	**2**	**2.7674**	**PHE123, HIS121,**	-
			khusimone	-8	-	-	-	-
OBP4	*Anopheles gambiae*	3Q8I	alpha-tocopherol	-8.83				-6.21
			alpha-vetivone	-8.15	2	2.87437	MET122, PHE121	-
			azardirachtin	**-16.48**	**2**	**3.0456**	**LEU89, SER109**	-
			gamma-sitosterol	-11.65	1	2.30465	SER109	-
			lycopersin	**-9.84**	**4**	**3.138158**	**MET122, GLN72,**	-
			saponin	-12.38	8	3.140481	ALA48, ALA106, SER9, LEU89, SER109, PHE121	-

OBP7	*Anopheles stephensi*	I-Tasser	**lycopersin**	**-8.42**	**6**	**2.605542**	**TYR29, HIS141, ILE69**	-5.84
			Phenol-2-Methoxy-3-(2-Propenyl)	-8.15	4	2.377353	HIS141, LYS138, CYS54, ILE137	-
OBP7	*Anopheles gambiae*	3R1P	alpha-cardinene	-8.47	-	-	-	-6.31
			alpha-longipipene	-8.45	-	-	-	-
			alpha-murolene	-8.35	-	-	-	-
			alpha-tocopherol	-10.38	-	-	-	-
			alpha-vetivone	-8.45	-	-	-	-
			azardirachtin	**-11.18**	**1**	**2.79207**	**VAL117**	-
			beta-vetivone	-8.89	1	3.62258	VAL117	-
			calarene	-8.93	-	-	-	-
			gamma-selenene	-8.28	-	-	-	-
			gamma-sitosterol	-10.28	-	-	-	-
			gurjunene	-8.38	-	-	-	-
			khusimol	**-8.5**	**1**	**1.95671**	**LEU32**	-
			khusimone	-8.32	-	-	-	-
			lycopersin	**-10.21**	**6**	**3.58058**	**PHE111, PHE120, PHE54,**	-
			saponin	-10.82	1	2.5775	LEU72	-
OBP20	*Anopheles gambiae*	3V2L	alpha-longipipene	-8.06	-	-	-	-6.6
			alpha-vetivone	-8.14	1	3.20328	SER66	-
			beta-humulene	-8.55	-	-	-	-
			beta-vetivone	-8.77	-	-	-	-

			gamma-selenene	-8.23	-	-	-	-
			gamma-sitosterol	-11.87	-	-	-	-
			khusimol	**-8.09**	**1**	**2.59997**	**MET74**	-
			khusimone	-8.53	-	-	-	-
			lycopersin	**-10.6**	**2**	**2.871935**	**ILE118, GLY10**	-
			Phenol-2-Methoxy-3-(2-Propenyl)	-8.16	-	-	-	-
OBP22A	*Anopheles gambiae*	3L4A	alpha-cardinene	-8.32	-	-	-	-6.32
			alpha-longipipene	-8.6	-	-	-	-
			alpha-murolene	-8.29	-	-	-	-
			alpha-tocopherol	-10.09	1	2.08183	VAL63	-
			alpha-vetivone	-8.32	-	-	-	-
			azardirachtin	**-20.09**	**5**	**3.31033**	**GLN136, GLN126, TYR122, ASN51,**	-
			beta-humulene	-8.06	-	-	-	-
			beta-vetivone	-8.32	1	3.08794	ARG129	-
			calacorene	-8.28	-	-	-	-
			calarene	-8.03	-	-	-	-
			gamma-selenene	-8.32	-	-	-	-
			gamma-sitosterol	-11.28	-	-	-	-
			gurjunene	-8.13	-	-	-	-
			khusimol	**-8.45**	**1**	**2.05531**	**PHE53**	-
			khusimone	-8.41	1	2.79075	GLN136,	-
			saponin	-10.76	6		ASN87, TYR135, ASN139, VAL63	-

			lycopersin	**-10.19**	**4**	**3.008718**	**PHE125, TYR122, VAL63**	-
OBP48	*Anopheles gambiae*	4IJ7	**azardirachtin**	**-12.05**	**2**	**3.165625**	**CYS54, LEU137**	-5.46
			gamma-sitosterol	-9.21	2	2.27662	LEU24, PROLINE22	-
			lycopersin	**-8.17**	**6**	**3.15462**	**LYS21, GLY141, ARG138, PRO22,**	-

* The common plant based compounds which targets most of the OBPs of *A. gambiae* and *A. stephensi* with higher binding energy that of DEET has been marked in bold.

Post docking analysis

Analysis of molecular interaction between OBPs and selected phytochemical compounds

Out of these 40 compounds few compounds were found to showing higher binding affinity (as compared to DEET) for all OBPs consisted in this study. Among these lycopersin, azadirachtin, khusimol show high binding affinity with more number of hydrophobic contacts.

1) Interaction of OBP1 (*Anopheles stephensi*) with azadirachtin and khusimol

The azadirachtin and khusimol exhibit binding energy -13.77 Kcal/mol and -8.05 Kcal/mol for OBP1 of *A. stephensi*. In case of OBP1, the azadirachtin exhibits 7 hydrogen bonds where Phe142, Val144, Ala107, Leu143 and His140 formed strong H-bonds. The interaction between khusimol and OBP1 obtained using LigPlot and PyMOL has been displayed in **Figure 4-5**. The detailed molecular interaction between lycopersin and azadirachtin with OBP1 has been summarized in **Table 7-8.**

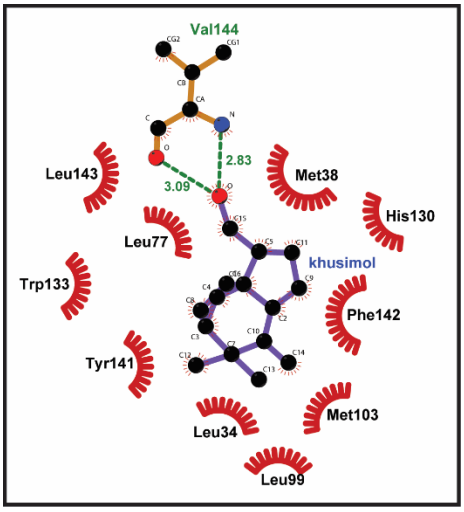

Figure 4 Molecular interaction of OBP1 with khusimol obtained using LigPlot.

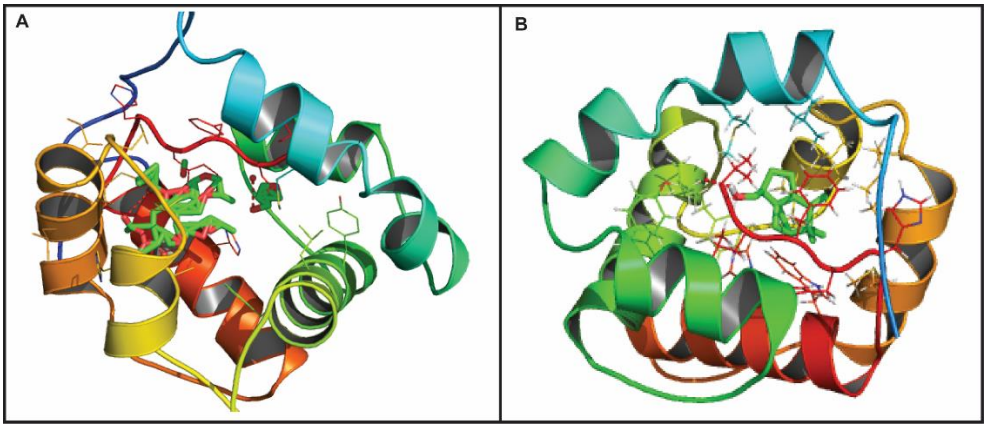

Figure 5 Molecular interaction of azadirachtin and khusimol with OBP1 using PyMOL.

Table 7 Detailed interaction analysis of OBP1 with lycopersin obtained using Discovery Studio Visualizer.

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:VAL144:H - :UNK0:O	1.89732	Hydrogen Bond	Conventional Hydrogen Bond	A:VAL144:H	H-Donor	:UNK0:O	H-Acceptor
:UNK0:O - A:PHE142:O	2.92538	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:PHE142:O	H-Acceptor
:UNK0:O - A:PHE142:O	2.48859	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:PHE142:O	H-Acceptor
A:HIS130:HE1 - :UNK0:O	2.80854	Hydrogen Bond	Carbon Hydrogen Bond	A:HIS130:HE1	H-Donor	:UNK0:O	H-Acceptor
A:HIS130:HE1 - :UNK0:O	2.49107	Hydrogen Bond	Carbon Hydrogen Bond	A:HIS130:HE1	H-Donor	:UNK0:O	H-Acceptor
A:LEU143:HA - :UNK0:O	2.08147	Hydrogen Bond	Carbon Hydrogen Bond	A:LEU143:HA	H-Donor	:UNK0:O	H-Acceptor
A:LEU143:HA - :UNK0:O	2.67259	Hydrogen Bond	Carbon Hydrogen Bond	A:LEU143:HA	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:VAL144:O	3.05571	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:VAL144:O	H-Acceptor
A:ALA107 - :UNK0	5.027	Hydrophobic	Alkyl	A:ALA107	Alkyl	:UNK0	Alkyl
A:ALA107 - :UNK0:C	4.03933	Hydrophobic	Alkyl	A:ALA107	Alkyl	:UNK0:C	Alkyl
A:MET110 - :UNK0	4.88031	Hydrophobic	Alkyl	A:MET110	Alkyl	:UNK0	Alkyl
:UNK0:C - A:LEU99	4.13296	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU99	Alkyl
:UNK0:C - A:MET103	4.21485	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:MET103	Alkyl

Table 8 Detailed interaction analysis of OBP1 with azadirachtin obtained using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:PHE142:H - :UNK0:O	2.81189	Hydrogen Bond	Conventional Hydrogen Bond	A:PHE142:H	H-Donor	:UNK0:O	H-Acceptor
A:VAL144:H - :UNK0:O	2.14822	Hydrogen Bond	Conventional Hydrogen Bond	A:VAL144:H	H-Donor	:UNK0:O	H-Acceptor
:UNK0:O - A:ALA107:O	2.53789	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:ALA107:O	H-Acceptor
A:ALA107:HA - :UNK0:O	1.80892	Hydrogen Bond	Carbon Hydrogen Bond	A:ALA107:HA	H-Donor	:UNK0:O	H-Acceptor
A:LEU143:HA - :UNK0:O	2.2335	Hydrogen Bond	Carbon Hydrogen Bond	A:LEU143:HA	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:HIS140:O	3.00592	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:HIS140:O	H-Acceptor
:UNK0:C - A:PHE142:O	3.39021	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:PHE142:O	H-Acceptor
:UNK0:O - A:TRP133	3.2075	Electrostatic	Pi-Cation	:UNK0:O	Positive	A:TRP133	Pi-Orbitals
:UNK0:O - A:HIS96	4.92354	Electrostatic	Pi-Cation	:UNK0:O	Positive	A:HIS96	Pi-Orbitals
:UNK0:O - A:TRP133	3.49723	Electrostatic	Pi-Cation	:UNK0:O	Positive	A:TRP133	Pi-Orbitals
:UNK0:O - A:TRP133	3.52956	Electrostatic	Pi-Cation	:UNK0:O	Positive	A:TRP133	Pi-Orbitals
:UNK0:O - A:TRP133	2.95191	Hydrogen Bond	Pi-Donor Hydrogen Bond	:UNK0:O	H-Donor	A:TRP133	Pi-Orbitals
:UNK0:C - A:TRP133	3.20334	Hydrophobic	Pi-Sigma	:UNK0:C	C-H	A:TRP133	Pi-Orbitals
A:ALA107 - :UNK0	3.14438	Hydrophobic	Alkyl	A:ALA107	Alkyl	:UNK0	Alkyl
:UNK0 - A:LEU99	5.42864	Hydrophobic	Alkyl	:UNK0	Alkyl	A:LEU99	Alkyl
:UNK0 - A:MET103	4.48783	Hydrophobic	Alkyl	:UNK0	Alkyl	A:MET103	Alkyl
:UNK0 - A:ILE106	5.47519	Hydrophobic	Alkyl	:UNK0	Alkyl	A:ILE106	Alkyl
:UNK0 - A:MET110	4.24034	Hydrophobic	Alkyl	:UNK0	Alkyl	A:MET110	Alkyl

2) Interaction of OBP7 (*Anopheles stephensi*) with lycopersin

Lycopersin comprised of a binding energy of -8.42 Kcal/mol withOBP7. Lycopersin exhibit 6 hydrogen bond with the residues Tyr29, His141 and Ile69 of OBP7. The interaction between lycopersin and OBP7 obtained using LigPlot and PyMOL has been displayed in **Figure 6-7**. The detailed molecular interaction between lycopersin with OBP7 has been summarized in **Table 9.**

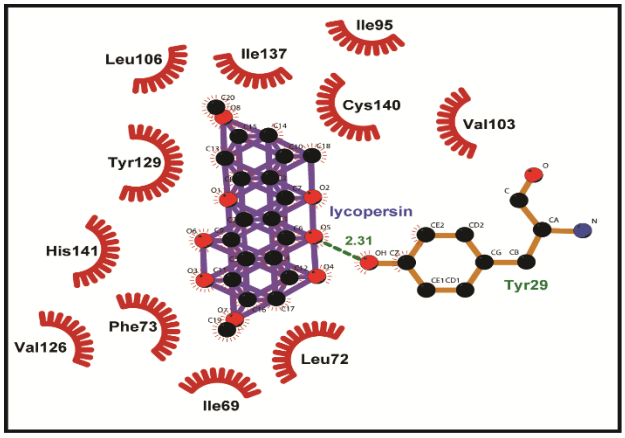

Figure 6 Molecular interaction of OBP7 with lycopersin obtained using LigPlot

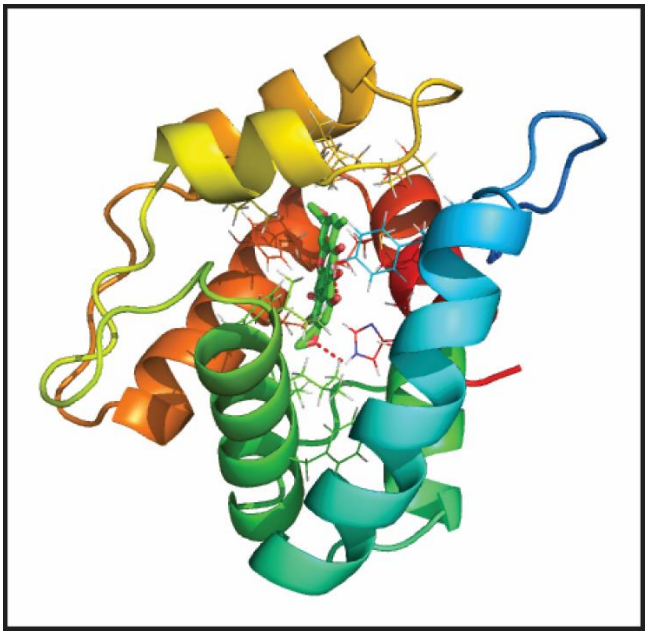

Figure 7 Molecular interaction of OBP7 with lycopersin obtained using PyMOL

Table 9 Detailed interaction analysis of OBP7 with lycopersin obtained using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:TYR29:HH - :UNK0:O	1.75647	Hydrogen Bond	Conventional Hydrogen Bond	A:TYR29:HH	H-Donor	:UNK0:O	H-Acceptor
A:TYR29:HH - :UNK0:O	2.03545	Hydrogen Bond	Conventional Hydrogen Bond	A:TYR29:HH	H-Donor	:UNK0:O	H-Acceptor
A:HIS141:HE2 - :UNK0:O	2.75318	Hydrogen Bond	Conventional Hydrogen Bond	A:HIS141:HE2	H-Donor	:UNK0:O	H-Acceptor
:UNK0:O - A:TYR29:OH	3.10772	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:TYR29:OH	H-Acceptor
A:HIS141:HE1 - :UNK0:O	2.94131	Hydrogen Bond	Carbon Hydrogen Bond	A:HIS141:HE1	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:ILE69:O	3.03912	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:ILE69:O	H-Acceptor
:UNK0:C - A:TYR129	3.75138	Hydrophobic	Pi-Sigma	:UNK0:C	C-H	A:TYR129	Pi-Orbitals
A:VAL103 - :UNK0	4.71054	Hydrophobic	Alkyl	A:VAL103	Alkyl	:UNK0	Alkyl
A:CYS140 - :UNK0	3.63549	Hydrophobic	Alkyl	A:CYS140	Alkyl	:UNK0	Alkyl
:UNK0:C - A:ILE95	5.10941	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:ILE95	Alkyl
:UNK0:C - A:VAL103	4.10448	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL103	Alkyl
:UNK0:C - A:CYS140	3.99727	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:CYS140	Alkyl

3) Interaction of OBP (*Anopheles gambiae*) with lycopersin, azadirachtin and khusimol

The OBP which comprised of a binding energy of -9.79, -10.05, -8.06 Kcal/mol. with lycopersin, azadirachtin, khusimol resp. Lycopersin exhibit 7 hydrogen bond with the residues Phe127, Ala139, Gly142 and Gly135 of OBP, azadirachtin exhibit 3 hydrogen bond with the residue Cys54, Thr21 and Phe172 of OBP and khusimol exhibit 2 hydrogen bond with the residues Leu24 and Val25 of OBP. The interaction between azadirachtin, khusimol and lycopersin with OBP obtained using LigPlot and PyMOL has been displayed in **Figure 8-9**. The detailed molecular interaction between lycopersin, azadirachtin and khusimol with OBP has been summarized in **Table 10-12** respectively.

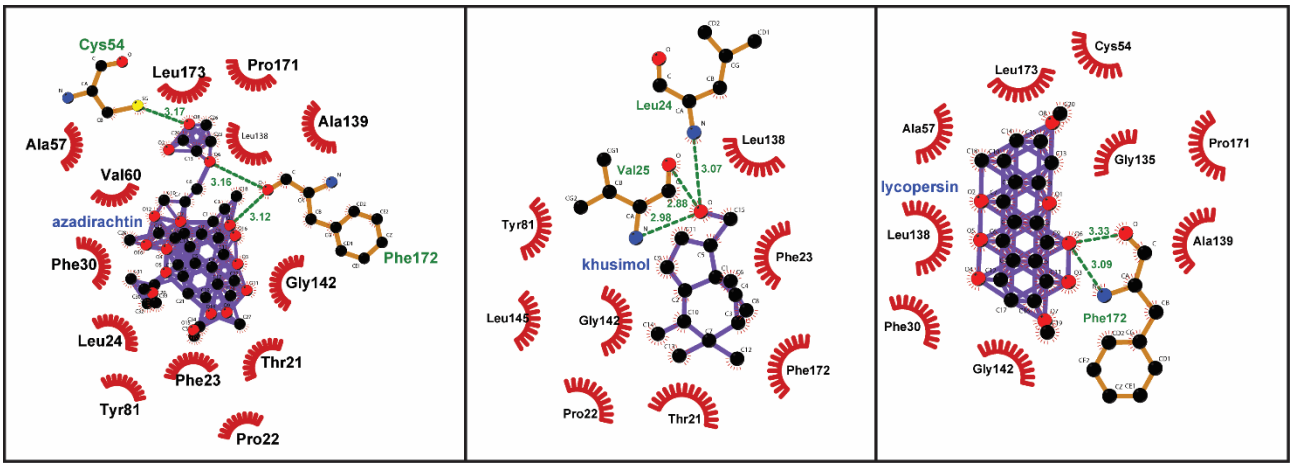

Figure 8 Molecular interaction of OBP with a zadirachtin, khusimol and lycopersin obtained using LigPlot.

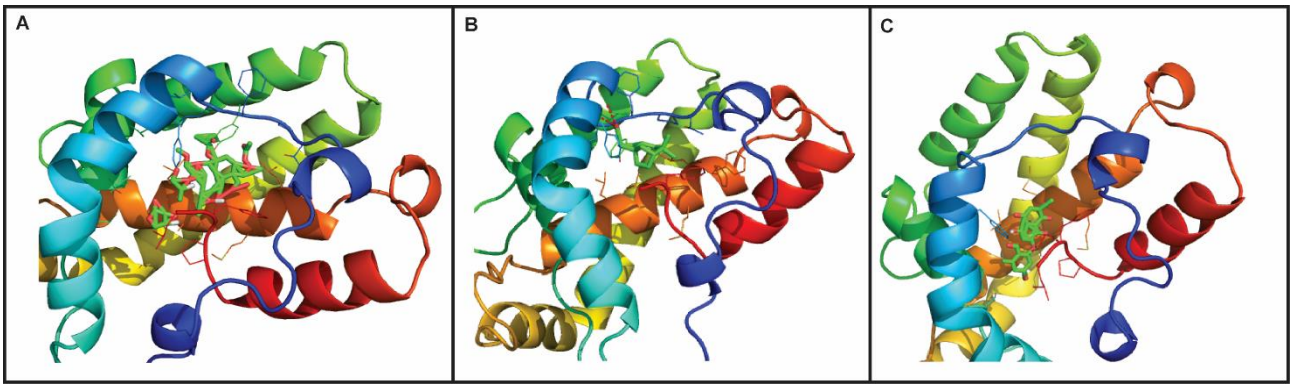

Figure 9 Molecular interaction of OBP with azadirachtin, khusimol and lycopersin obtained using PyMOL.

Table 10 Interaction between OBP from *Anopheles gambiae* with lycopersin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:PHE172:N - :UNK0:O	3.09408	Hydrogen Bond	Conventional Hydrogen Bond	A:PHE172:N	H-Donor	:UNK0:O	H-Acceptor
A:ALA139:CA - :UNK0:O	3.11121	Hydrogen Bond	Carbon Hydrogen Bond	A:ALA139:CA	H-Donor	:UNK0:O	H-Acceptor
A:ALA139:CA - :UNK0:O	3.32722	Hydrogen Bond	Carbon Hydrogen Bond	A:ALA139:CA	H-Donor	:UNK0:O	H-Acceptor
A:GLY142:CA - :UNK0:O	2.69868	Hydrogen Bond	Carbon Hydrogen Bond	A:GLY142:CA	H-Donor	:UNK0:O	H-Acceptor
A:GLY142:CA - :UNK0:O	2.86394	Hydrogen Bond	Carbon Hydrogen Bond	A:GLY142:CA	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:PHE172:O	3.06541	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:PHE172:O	H-Acceptor
:UNK0:C - A:GLY135:O	3.54086	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:GLY135:O	H-Acceptor
A:CYS54 - :UNK0	4.4517	Hydrophobic	Alkyl	A:CYS54	Alkyl	:UNK0	Alkyl
A:ALA57 - :UNK0	4.63884	Hydrophobic	Alkyl	A:ALA57	Alkyl	:UNK0	Alkyl
A:ALA57 - :UNK0:C	3.41927	Hydrophobic	Alkyl	A:ALA57	Alkyl	:UNK0:C	Alkyl
A:LEU138 - :UNK0	5.28835	Hydrophobic	Alkyl	A:LEU138	Alkyl	:UNK0	Alkyl
A:ALA139 - :UNK0	4.00125	Hydrophobic	Alkyl	A:ALA139	Alkyl	:UNK0	Alkyl
A:PRO171 - :UNK0	4.63199	Hydrophobic	Alkyl	A:PRO171	Alkyl	:UNK0	Alkyl
A:LEU173 - :UNK0	5.12104	Hydrophobic	Alkyl	A:LEU173	Alkyl	:UNK0	Alkyl
:UNK0:C - A:CYS54	4.13991	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:CYS54	Alkyl
:UNK0:C - A:VAL56	4.33313	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL56	Alkyl
:UNK0:C - A:LEU138	4.65421	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU138	Alkyl

Table 11 Interaction between OBP from *Anopheles gambiae* with azadirachtin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:CYS54:SG - :UNK0:O	3.16855	Hydrogen Bond	Conventional Hydrogen Bond	A:CYS54:SG	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:THR21:OG1	2.69558	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:THR21:OG1	H-Acceptor
:UNK0:O - A:PHE172	3.84658	Hydrogen Bond	Pi-Donor Hydrogen Bond	:UNK0:O	H-Donor	A:PHE172	Pi-Orbitals
A:VAL25 - :UNK0	5.08125	Hydrophobic	Alkyl	A:VAL25	Alkyl	:UNK0	Alkyl
:UNK0 - A:LEU24	4.64389	Hydrophobic	Alkyl	:UNK0	Alkyl	A:LEU24	Alkyl
:UNK0 - A:LEU138	4.96892	Hydrophobic	Alkyl	:UNK0	Alkyl	A:LEU138	Alkyl
:UNK0:C - A:LEU24	4.43819	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU24	Alkyl
:UNK0:C - A:LEU138	5.23058	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU138	Alkyl
A:TYR81 - :UNK0:C	5.02722	Hydrophobic	Pi-Alkyl	A:TYR81	Pi-Orbitals	:UNK0:C	Alkyl

Table 12 Interaction between OBP from *Anopheles gambiae* with khusimol using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:LEU24:N - :UNK0:O	3.06793	Hydrogen Bond	Conventional Hydrogen Bond	A:LEU24:N	H-Donor	:UNK0:O	H-Acceptor
:UNK0:H - A:VAL25:O	2.00487	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:H	H-Donor	A:VAL25:O	H-Acceptor
:UNK0:C - A:PHE172	3.75569	Hydrophobic	Pi-Sigma	:UNK0:C	C-H	A:PHE172	Pi-Orbitals
:UNK0 - A:LEU24	5.20232	Hydrophobic	Alkyl	:UNK0	Alkyl	A:LEU24	Alkyl
:UNK0:C - A:LEU145	4.16619	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU145	Alkyl
A:TYR81 - :UNK0	5.0913	Hydrophobic	Pi-Alkyl	A:TYR81	Pi-Orbitals	:UNK0	Alkyl

4) Interaction of OBP1 (*Anopheles gambiae*) with lycopersin, azadirachtin and khusimol

The OBP1 which comprised of a binding energy of -10.42, -12.33, -8.16 Kcal/mol with lycopersin, azadirachtin, khusimol resp. Lycopersin exhibit 8 hydrogen bond with the residues Val125, Phe123, His111, Tyr122, Tyr10, His121 and Phe123 of OBP1. Azadirachtin exhibit 3 hydrogen bond with the residue Leu124, Val125 and Ser79 of OBP1 and khusimol exhibit 2 hydrogen bond with the residues Phe123 and His121 of OBP1.The interaction between lycopersin, azadirachtin and khusimol with OBP1 obtained using LigPlot and PyMOL has been displayed in **Figure 10-11**. The detailed molecular interaction between lycopersin, khusimol and azadirachtin with OBP1 has been summarized in **Table 13-15.**

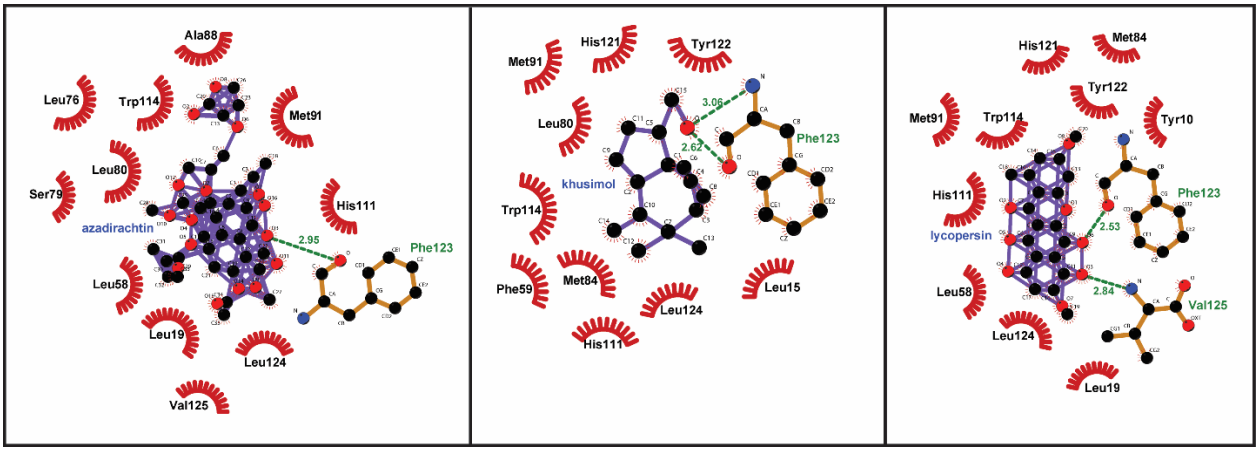

Figure 10 Molecular interaction of OBP1 with azadirachtin, khusimol and lycopersin obtained using LigPlot

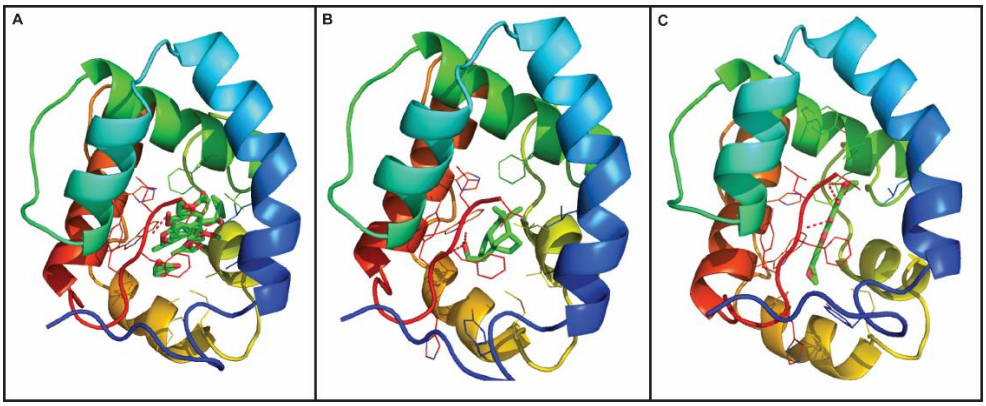

Figure 11 Molecular interaction of OBP1 with azadirachtin, khusimol and lycopersin obtained using PyMOL

Table 13 Interaction between OBP1 from *Anopheles gambiae* with lycopersin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:VAL125:N - :UNK0:O	2.84415	Hydrogen Bond	Conventional Hydrogen Bond	A:VAL125:N	H-Donor	:UNK0:O	H-Acceptor
:UNK0:O - A:PHE123:O	2.52509	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:PHE123:O	H-Acceptor
A:HIS111:CE1 - :UNK0:O	3.51929	Hydrogen Bond	Carbon Hydrogen Bond	A:HIS111:CE1	H-Donor	:UNK0:O	H-Acceptor
A:HIS111:CE1 - :UNK0:O	3.27224	Hydrogen Bond	Carbon Hydrogen Bond	A:HIS111:CE1	H-Donor	:UNK0:O	H-Acceptor
A:TYR122:CA - :UNK0:O	3.51809	Hydrogen Bond	Carbon Hydrogen Bond	A:TYR122:CA	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:TYR10:OH	3.25302	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:TYR10:OH	H-Acceptor
:UNK0:C - A:HIS121:O	3.10769	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:HIS121:O	H-Acceptor
:UNK0:O - A:PHE123	3.4676	Hydrogen Bond	Pi-Donor Hydrogen Bond	:UNK0:O	H-Donor	A:PHE123	Pi-Orbitals
:UNK0:C - A:TRP114	3.7132	Hydrophobic	Pi-Sigma	:UNK0:C	C-H	A:TRP114	Pi-Orbitals
:UNK0:C - A:MET91	5.49045	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:MET91	Alkyl
A:TRP114 - :UNK0:C	4.6789	Hydrophobic	Pi-Alkyl	A:TRP114	Pi-Orbitals	:UNK0:C	Alkyl
A:TYR122 - :UNK0	5.05558	Hydrophobic	Pi-Alkyl	A:TYR122	Pi-Orbitals	:UNK0	Alkyl
A:PHE123 - :UNK0	4.5705	Hydrophobic	Pi-Alkyl	A:PHE123	Pi-Orbitals	:UNK0	Alkyl

Table 14 Interaction between OBP1 from *Anopheles gambiae* with azadirachtin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
:UNK0:O - A:VAL125:O	4.39382	Electrostatic	Attractive Charge	:UNK0:O	Positive	A:VAL125:O	Negative
A:LEU124:CA - :UNK0:O	3.03169	Hydrogen Bond	Carbon Hydrogen Bond	A:LEU124:CA	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:VAL125:O	3.24738	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:VAL125:O	H-Acceptor
:UNK0:C - A:SER79:OG	3.18792	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:SER79:OG	H-Acceptor
:UNK0:C - A:TRP114	3.70215	Hydrophobic	Pi-Sigma	:UNK0:C	C-H	A:TRP114	Pi-Orbitals

A:ALA62 - :UNK0	4.19992	Hydrophobic	Alkyl	A:ALA62	Alkyl	:UNK0	Alkyl
A:ALA62 - :UNK0:C	4.2316	Hydrophobic	Alkyl	A:ALA62	Alkyl	:UNK0:C	Alkyl
:UNK0:C - A:MET91	4.81462	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:MET91	Alkyl
:UNK0 - A:LEU19	5.36015	Hydrophobic	Alkyl	:UNK0	Alkyl	A:LEU19	Alkyl
:UNK0:C - A:LEU19	5.39875	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU19	Alkyl
:UNK0:C - A:LEU58	4.45672	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU58	Alkyl
A:PHE59 - :UNK0:C	5.2231	Hydrophobic	Pi-Alkyl	A:PHE59	Pi-Orbitals	:UNK0:C	Alkyl
A:TRP114 - :UNK0:C	4.32294	Hydrophobic	Pi-Alkyl	A:TRP114	Pi-Orbitals	:UNK0:C	Alkyl
A:TYR122 - :UNK0:C	5.23034	Hydrophobic	Pi-Alkyl	A:TYR122	Pi-Orbitals	:UNK0:C	Alkyl

Table 15 Interaction between OBP1 from *Anopheles gambiae* with kusimol using Discovery Studio Visualizer

Interaction	**Distance**	**Category**	**Type**	**From**	**From Chemistry**	**To**	**To Chemistry**
:UNK0:H - A:PHE123:O	1.74502	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:H	H-Donor	A:PHE123:O	H-Acceptor
:UNK0:C - A:HIS121:O	3.78978	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:HIS121:O	H-Acceptor
:UNK0:C - A:LEU124	4.35089	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU124	Alkyl
:UNK0:C - A:LEU76	5.01137	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU76	Alkyl
A:PHE59 - :UNK0:C	4.82958	Hydrophobic	Pi-Alkyl	A:PHE59	Pi-Orbitals	:UNK0:C	Alkyl
A:HIS111 - :UNK0:C	4.36413	Hydrophobic	Pi-Alkyl	A:HIS111	Pi-Orbitals	:UNK0:C	Alkyl
A:HIS111 - :UNK0:C	4.41559	Hydrophobic	Pi-Alkyl	A:HIS111	Pi-Orbitals	:UNK0:C	Alkyl
A:TRP114 - :UNK0	5.22612	Hydrophobic	Pi-Alkyl	A:TRP114	Pi-Orbitals	:UNK0	Alkyl
A:TRP114 - :UNK0	4.27656	Hydrophobic	Pi-Alkyl	A:TRP114	Pi-Orbitals	:UNK0	Alkyl
A:TRP114 - :UNK0:C	4.33072	Hydrophobic	Pi-Alkyl	A:TRP114	Pi-Orbitals	:UNK0:C	Alkyl
A:PHE123 - :UNK0	4.02237	Hydrophobic	Pi-Alkyl	A:PHE123	Pi-Orbitals	:UNK0	Alkyl

5) Interaction of OBP4 (*Anopheles gambiae*) with lycopersin, azadirachtin

The OBP4 which comprised of a binding energy of -9.84, -16.48 Kcal/mol with lycopersin, azadirachtin resp. Lycopersin exhibit 4 hydrogen bond with the residues MET122 and GLN72 of OBP4, azadirachtin exhibit 2 hydrogen bond with the residue LEU89 and SER109 of OBP4. The interaction between lycopersin, azadirachtin with OBP4 obtained using LigPlot and PyMOL has been displayed in **Figure 12-13**. The detailed molecular interaction between lycopersin, khusimol and azadirachtin with OBP4 has been summarized in **Table 16 - 17**.

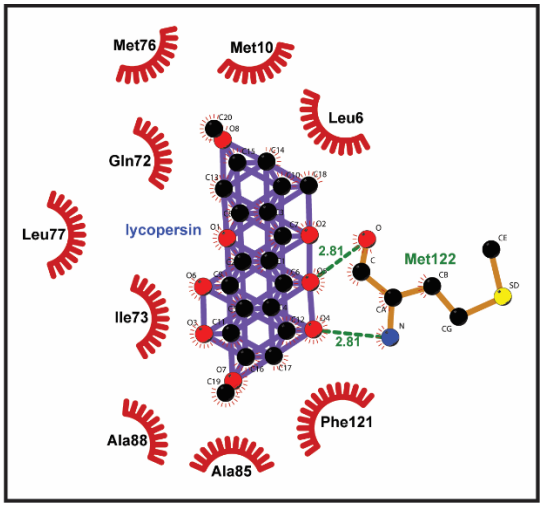

Figure 12 Molecular interaction between OBP4 and lycopersin obtained using ligplot

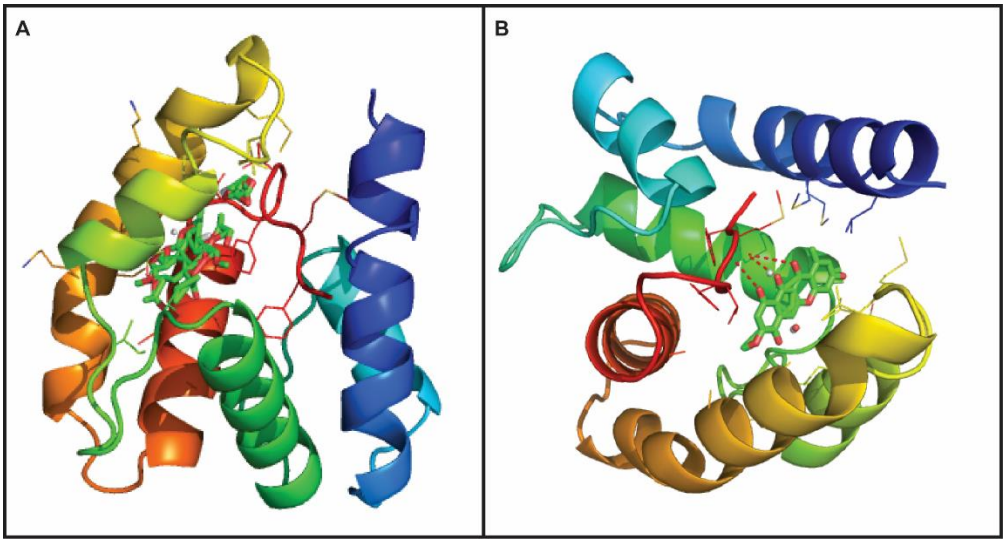

Figure 13 Molecular interaction of OBP4 with lycopersin and azadirachtin obtained using PyMOL

Table 16 Interaction between OBP4 from *Anopheles gambiae* with lycopersin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:MET122:N - :UNK0:O	2.80674	Hydrogen Bond	Conventional Hydrogen Bond	A:MET122:N	H-Donor	:UNK0:O	H-Acceptor
:UNK0:O - A:MET122:O	3.33182	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:MET122:O	H-Acceptor
:UNK0:O - A:MET122:O	2.81264	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:MET122:O	H-Acceptor
:UNK0:C - A:GLN72:OE1	3.60143	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:GLN72:OE1	H-Acceptor
A:LEU6 - :UNK0	5.42907	Hydrophobic	Alkyl	A:LEU6	Alkyl	:UNK0	Alkyl
A:MET76 - :UNK0	5.03995	Hydrophobic	Alkyl	A:MET76	Alkyl	:UNK0	Alkyl
A:LEU77 - :UNK0	5.09375	Hydrophobic	Alkyl	A:LEU77	Alkyl	:UNK0	Alkyl
:UNK0:C - A:LEU6	4.25796	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU6	Alkyl
:UNK0:C - A:MET10	3.79559	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:MET10	Alkyl

Table 17 Interaction between OBP4 from *Anopheles gambiae* with azadirachtin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:LEU89:N - :UNK0:O	3.33651	Hydrogen Bond	Conventional Hydrogen Bond	A:LEU89:N	H-Donor	:UNK0:O	H-Acceptor
A:SER109:CB - :UNK0:O	2.75469	Hydrogen Bond	Carbon Hydrogen Bond	A:SER109:CB	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:ILE64	4.33542	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:ILE64	Alkyl

6) Interaction of OBP7 (*Anopheles gambiae*) with lycopersin, azadirachtin and khusimol

The OBP7 which comprised of a binding energy of -10.21, -11.18, -8.5 Kcal/mol. with lycopersin, azadirachtin and khusimol resp. Lycopersin exhibit 6 hydrogen bond with the residues Phe111, Phe120, Phe54 of OBP7, azadirachtin exhibit 1 hydrogen bond with the residue Val117 of OBP7 and khusimol exhibit 1 hydrogen bond with the residue Leu32 of OBP7. The interaction between lycopersin, azadirachtin and khusimol with OBP7 obtained using LigPlot and PyMOL has been displayed in **Figure 14-15**. The detailed molecular interaction between lycopersin, khusimol and azadirachtin with OBP7 has been summarized in **Table 18-20.**

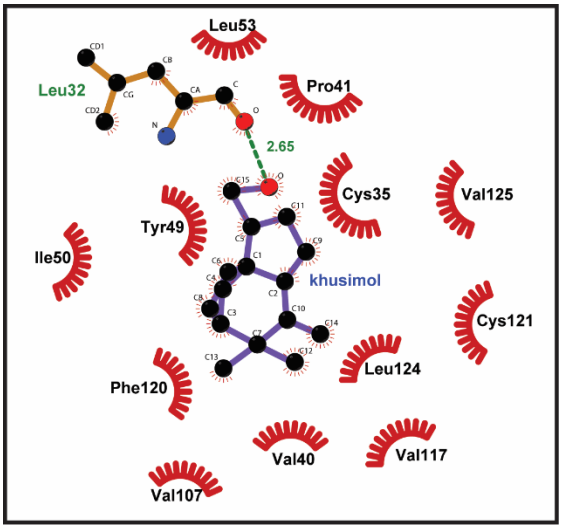

Figure 14 Molecular interaction between OBP7 and khusimol obtained using ligplot

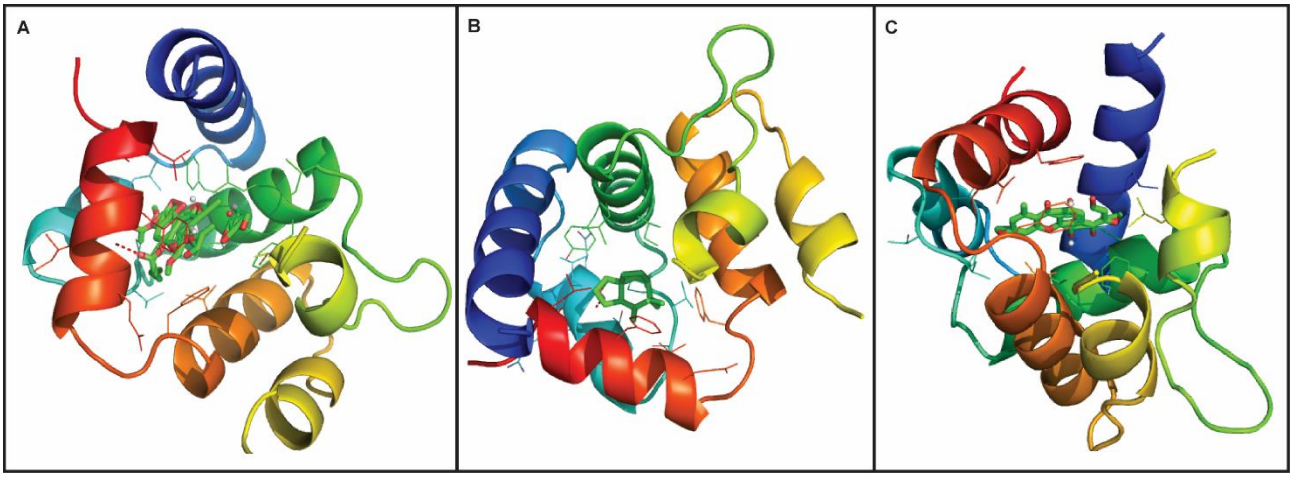

Figure 15 Molecular interaction of OBP7 with azadirachtin, khusimol and lycopersin obtained using PyMOL

Table 18 Interaction between OBP7 from *Anopheles gambiae* with lycopersin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
:UNK0:O - A:PHE111	3.5189	Hydrogen Bond	Pi-Donor Hydrogen Bond	:UNK0:O	H-Donor	A:PHE111	Pi-Orbitals
:UNK0:O - A:PHE120	3.59301	Hydrogen Bond	Pi-Donor Hydrogen Bond	:UNK0:O	H-Donor	A:PHE120	Pi-Orbitals
:UNK0:O - A:PHE54	3.87564	Hydrogen Bond	Pi-Donor Hydrogen Bond	:UNK0:O	H-Donor	A:PHE54	Pi-Orbitals
:UNK0:O - A:PHE120	3.60637	Hydrogen Bond	Pi-Donor Hydrogen Bond	:UNK0:O	H-Donor	A:PHE120	Pi-Orbitals
:UNK0:O - A:PHE111	3.71502	Hydrogen Bond	Pi-Donor Hydrogen Bond	:UNK0:O	H-Donor	A:PHE111	Pi-Orbitals
:UNK0:O - A:PHE120	3.17454	Hydrogen Bond	Pi-Donor Hydrogen Bond	:UNK0:O	H-Donor	A:PHE120	Pi-Orbitals
A:VAL40 - :UNK0	5.06116	Hydrophobic	Alkyl	A:VAL40	Alkyl	:UNK0	Alkyl
A:VAL107 - :UNK0	4.02649	Hydrophobic	Alkyl	A:VAL107	Alkyl	:UNK0	Alkyl
A:VAL117 - :UNK0	4.55008	Hydrophobic	Alkyl	A:VAL117	Alkyl	:UNK0	Alkyl
:UNK0:C - A:VAL107	5.05654	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL107	Alkyl
:UNK0:C - A:VAL117	3.90737	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL117	Alkyl
A:PHE111 - :UNK0	5.39119	Hydrophobic	Pi-Alkyl	A:PHE111	Pi-Orbitals	:UNK0	Alkyl
A:PHE111 - :UNK0:C	4.18013	Hydrophobic	Pi-Alkyl	A:PHE111	Pi-Orbitals	:UNK0:C	Alkyl
A:PHE120 - :UNK0:C	4.73721	Hydrophobic	Pi-Alkyl	A:PHE120	Pi-Orbitals	:UNK0:C	Alkyl

Table 19 Interaction between OBP from *Anopheles gambiae* with azadirachtin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
:UNK0:O - A:VAL117:O	2.79207	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:VAL117:O	H-Acceptor
:UNK0:O - A:PHE111	4.61445	Electrostatic	Pi-Cation	:UNK0:O	Positive	A:PHE111	Pi-Orbitals
:UNK0:O - A:PHE111	4.59879	Electrostatic	Pi-Cation	:UNK0:O	Positive	A:PHE111	Pi-Orbitals
:UNK0:O - A:PHE120	3.46769	Electrostatic	Pi-Cation	:UNK0:O	Positive	A:PHE120	Pi-Orbitals
:UNK0:C - A:PHE111	2.9898	Hydrophobic	Pi-Sigma	:UNK0:C	C-H	A:PHE111	Pi-Orbitals
:UNK0:O - A:PHE120	2.9608	Other	Pi-Lone Pair	:UNK0:O	Lone Pair	A:PHE120	Pi-Orbitals
:UNK0 - A:VAL40	4.18095	Hydrophobic	Alkyl	:UNK0	Alkyl	A:VAL40	Alkyl
:UNK0 - A:VAL107	4.21138	Hydrophobic	Alkyl	:UNK0	Alkyl	A:VAL107	Alkyl
:UNK0 - A:VAL117	3.2397	Hydrophobic	Alkyl	:UNK0	Alkyl	A:VAL117	Alkyl
:UNK0:C - A:VAL40	3.34371	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL40	Alkyl
:UNK0:C - A:PRO41	5.37705	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:PRO41	Alkyl
:UNK0:C - A:VAL107	3.9402	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL107	Alkyl
:UNK0:C - A:VAL117	4.17413	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL117	Alkyl
A:PHE111 - :UNK0	5.40941	Hydrophobic	Pi-Alkyl	A:PHE111	Pi-Orbitals	:UNK0	Alkyl

Table 20 Interaction between OBP7 from *Anopheles gambiae* with khusimol using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
:UNK0:H - A:LEU32:O	1.95671	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:H	H-Donor	A:LEU32:O	H-Acceptor
A:CYS35 - :UNK0	3.91058	Hydrophobic	Alkyl	A:CYS35	Alkyl	:UNK0	Alkyl
A:PRO41 - :UNK0	5.35186	Hydrophobic	Alkyl	A:PRO41	Alkyl	:UNK0	Alkyl
A:ILE50 - :UNK0	3.99892	Hydrophobic	Alkyl	A:ILE50	Alkyl	:UNK0	Alkyl
A:VAL107 - :UNK0	5.04027	Hydrophobic	Alkyl	A:VAL107	Alkyl	:UNK0	Alkyl
A:CYS121 - :UNK0	4.72639	Hydrophobic	Alkyl	A:CYS121	Alkyl	:UNK0	Alkyl
A:VAL125 - :UNK0	5.20994	Hydrophobic	Alkyl	A:VAL125	Alkyl	:UNK0	Alkyl
:UNK0 - A:LEU124	5.00498	Hydrophobic	Alkyl	:UNK0	Alkyl	A:LEU124	Alkyl
:UNK0:C - A:VAL40	3.41143	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL40	Alkyl
:UNK0:C - A:PRO41	4.68355	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:PRO41	Alkyl
:UNK0:C - A:VAL107	5.12235	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL107	Alkyl
:UNK0:C - A:VAL117	3.66662	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL117	Alkyl
:UNK0:C - A:VAL107	4.11979	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL107	Alkyl
:UNK0:C - A:VAL117	4.11483	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL117	Alkyl
:UNK0:C - A:VAL117	4.43869	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL117	Alkyl
:UNK0:C - A:CYS121	4.79	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:CYS121	Alkyl
A:PHE111 - :UNK0:C	5.21967	Hydrophobic	Pi-Alkyl	A:PHE111	Pi-Orbitals	:UNK0:C	Alkyl

7) Interaction of OBP20 (*Anopheles gambiae*) with lycopersin and khusimol

The OBP20which comprised of a binding energy of -10.6, -8.09 Kcal/mol with lycopersin and khusimol resp. Lycopersin exhibit 2 hydrogen bond with the residues Ile118 and Gly10 of OBP20, and khusimol exhibit 1 hydrogen bond with the residue Met74 of OBP20. The interaction between lycopersin and khusimol with OBP20 obtained using LigPlot and PyMOL has been displayed in **Figure 16-17**. The detailed molecular interaction between lycopersin and khusimol with OBP20 has been summarized in **Table 21-22.**

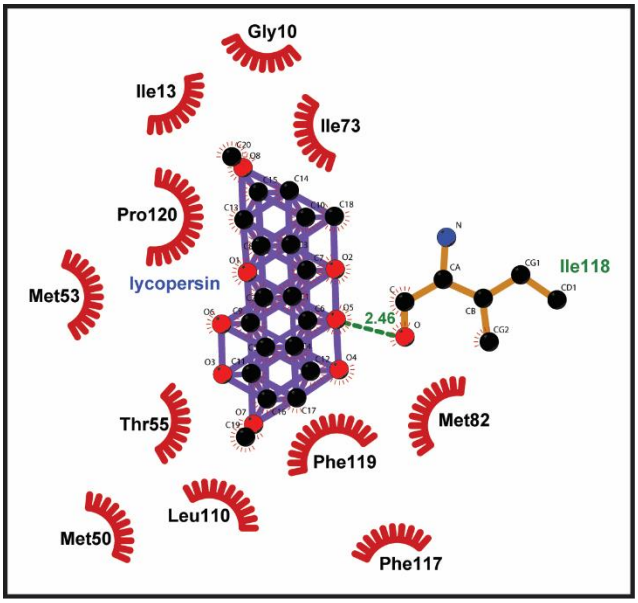

Figure 16 Molecular interaction between OBP20 and lycopersin obtained using ligplot

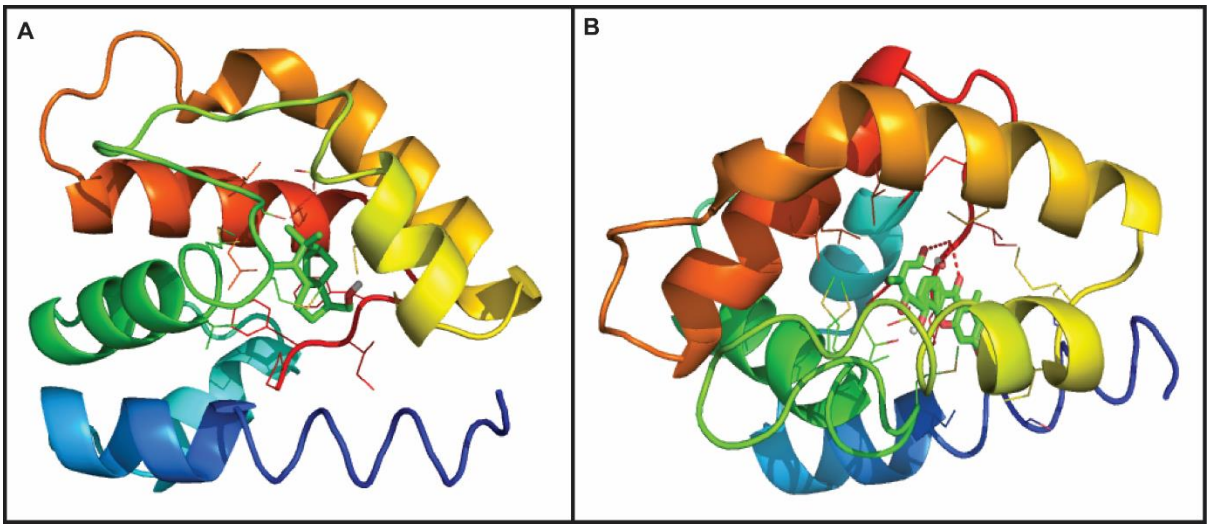

Figure 17 Molecular interaction of OBP20 with khusimol and lycopersin using PyMOL

Table 21 Interaction between OBP20 from *Anopheles gambiae* with lycopersin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
:UNK0:O - A:ILE118:O	2.45609	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:ILE118:O	H-Acceptor
A:GLY10:CA - :UNK0:O	3.28778	Hydrogen Bond	Carbon Hydrogen Bond	A:GLY10:CA	H-Donor	:UNK0:O	H-Acceptor
A:MET50:SD:B - :UNK0:O	2.94365	Other	Sulfur-X	A:MET50:SD:B	Sulfur	:UNK0:O	O,N,S
A:MET53 - :UNK0	4.79412	Hydrophobic	Alkyl	A:MET53	Alkyl	:UNK0	Alkyl
A:PRO120 - :UNK0	3.46159	Hydrophobic	Alkyl	A:PRO120	Alkyl	:UNK0	Alkyl
:UNK0:C - A:ILE118	4.14736	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:ILE118	Alkyl
:UNK0:C - A:PRO120	4.36293	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:PRO120	Alkyl

Table 22 Interaction between OBP20 from *Anopheles gambiae* with khusimol using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
:UNK0:H - A:MET74:SD	2.59997	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:H	H-Donor	A:MET74:SD	H-Acceptor
A:MET82 - :UNK0	3.80708	Hydrophobic	Alkyl	A:MET82	Alkyl	:UNK0	Alkyl
A:LEU110 - :UNK0	4.95426	Hydrophobic	Alkyl	A:LEU110	Alkyl	:UNK0	Alkyl
A:PRO120 - :UNK0	4.59333	Hydrophobic	Alkyl	A:PRO120	Alkyl	:UNK0	Alkyl
:UNK0 - A:MET53	4.07345	Hydrophobic	Alkyl	:UNK0	Alkyl	A:MET53	Alkyl
:UNK0:C - A:MET53	4.81711	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:MET53	Alkyl
:UNK0:C - A:ILE70	4.14348	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:ILE70	Alkyl
:UNK0:C - A:MET50	4.41768	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:MET50	Alkyl
:UNK0:C - A:LEU110	5.06446	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU110	Alkyl
:UNK0:C - A:MET50	4.10034	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:MET50	Alkyl
:UNK0:C - A:MET53	3.79693	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:MET53	Alkyl
A:PHE119 - :UNK0	4.69023	Hydrophobic	Pi-Alkyl	A:PHE119	Pi-Orbitals	:UNK0	Alkyl
A:PHE119 - :UNK0:C	5.26813	Hydrophobic	Pi-Alkyl	A:PHE119	Pi-Orbitals	:UNK0:C	Alkyl

8) Interaction of OBP22a (*Anopheles gambiae*) with lycopersin, azadirachtin and khusimol

The OBP22awhich comprised of a binding energy of -10.19, -20.09, -8.45 Kcal/mol with lycopersin, azadirachtin and khusimol resp. Lycopersin exhibit 4 hydrogen bond with the residues Phe125, Tyr122 and Val63 of OBP22a, azadirachtin exhibit 5 hydrogen bond with the residue Gln136, Gln126, Tyr122 and Asn51 of OBP22a and khusimol exhibit 1 hydrogen bond with the residue PHE53 of OBP22a. The interaction between khusimol with OBP22a obtained using LigPlot and PyMOL has been displayed in **Figure 18-19.** The detailed molecular interaction between lycopersin, khusimol and azadirachtin with OBP22a has been summarized in **Table 23-25.** .

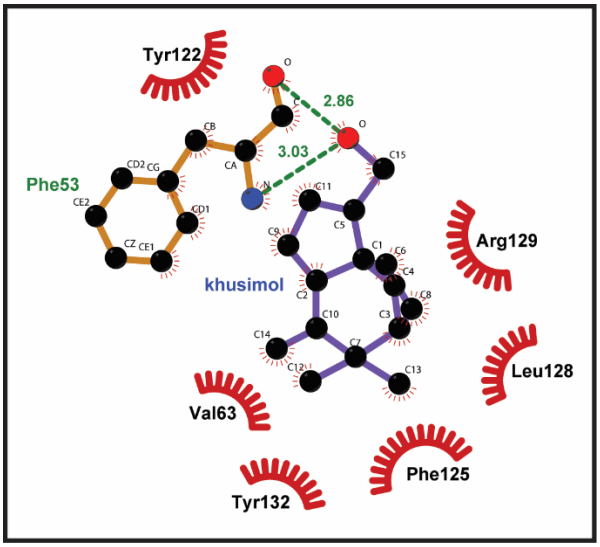

Figure 18: Molecular interaction of OBP22a with khusimol obtained using ligplot

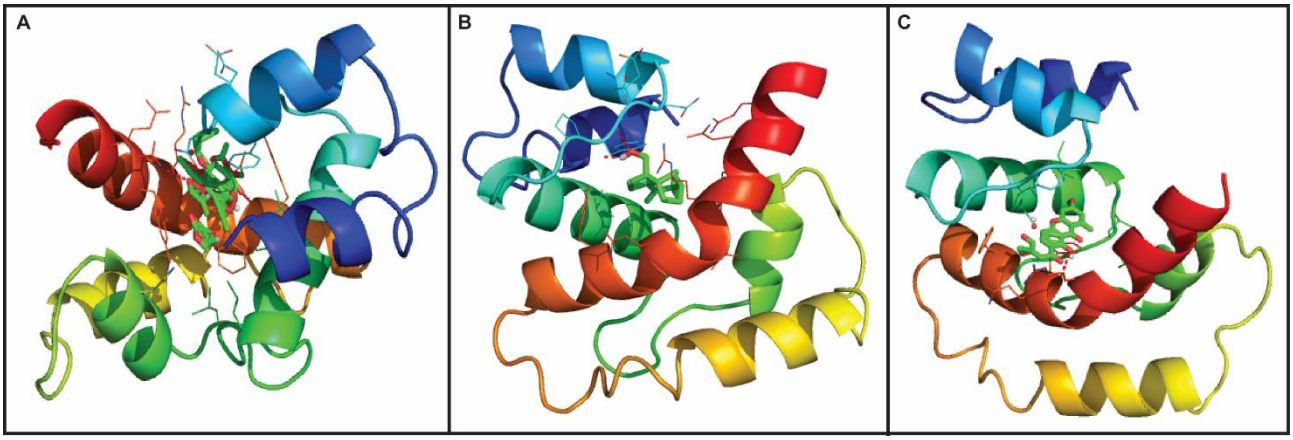

Figure 19: Molecular interaction of OBP22a with azadirachtin, khusimol and lycopersin using PyMOL

Table 23 Interaction between OBP22a from *Anopheles gambiae* with lycopersin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
:UNK0:O - A:PHE125:O	2.63244	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:PHE125:O	H-Acceptor
:UNK0:O - A:PHE125:O	2.90478	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:PHE125:O	H-Acceptor
:UNK0:C - A:TYR122:OH	3.24128	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:TYR122:OH	H-Acceptor
:UNK0:C - A:VAL63:O	3.25637	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:VAL63:O	H-Acceptor
A:VAL63 - :UNK0	4.55738	Hydrophobic	Alkyl	A:VAL63	Alkyl	:UNK0	Alkyl
A:PHE125 - :UNK0	3.9885	Hydrophobic	Pi-Alkyl	A:PHE125	Pi-Orbitals	:UNK0	Alkyl
A:PHE125 - :UNK0:C	4.20964	Hydrophobic	Pi-Alkyl	A:PHE125	Pi-Orbitals	:UNK0:C	Alkyl
A:TYR132 - :UNK0:C	3.66391	Hydrophobic	Pi-Alkyl	A:TYR132	Pi-Orbitals	:UNK0:C	Alkyl

Table 24 Interaction between OBP22a from *Anopheles gambiae* with azadirachtin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:ARG129:NH1 - :UNK0:O	5.47253	Electrostatic	Attractive Charge	A:ARG129:NH1	Positive	:UNK0:O	Negative
A:GLN136:NE2 - :UNK0:O	3.09568	Hydrogen Bond	Conventional Hydrogen Bond	A:GLN136:NE2	H-Donor	:UNK0:O	H-Acceptor
A:GLN126:CA - :UNK0:O	3.56338	Hydrogen Bond	Carbon Hydrogen Bond	A:GLN126:CA	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:TYR122:O	3.15462	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:TYR122:O	H-Acceptor
:UNK0:C - A:ASN51:O	3.60162	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:ASN51:O	H-Acceptor
:UNK0:C - A:GLN136:OE1	3.13635	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:GLN136:OE1	H-Acceptor
:UNK0:O - A:TYR132	4.48651	Electrostatic	Pi-Cation	:UNK0:O	Positive	A:TYR132	Pi-Orbitals
:UNK0:O - A:TYR122	4.60748	Electrostatic	Pi-Cation	:UNK0:O	Positive	A:TYR122	Pi-Orbitals
:UNK0:C - A:TYR122	3.22848	Hydrophobic	Pi-Sigma	:UNK0:C	C-H	A:TYR122	Pi-Orbitals
A:ARG129 - :UNK0	4.73843	Hydrophobic	Alkyl	A:ARG129	Alkyl	:UNK0	Alkyl
A:PHE53 - :UNK0	5.34966	Hydrophobic	Pi-Alkyl	A:PHE53	Pi-Orbitals	:UNK0	Alkyl

Table 25 Interaction between OBP22a from *Anopheles gambiae* with khusimol using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
:UNK0:H - A:PHE53:O	2.05531	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:H	H-Donor	A:PHE53:O	H-Acceptor
:UNK0:C - A:TYR132	3.65264	Hydrophobic	Pi-Sigma	:UNK0:C	C-H	A:TYR132	Pi-Orbitals
A:VAL63 - :UNK0	4.83725	Hydrophobic	Alkyl	A:VAL63	Alkyl	:UNK0	Alkyl
A:ARG129 - :UNK0	4.33485	Hydrophobic	Alkyl	A:ARG129	Alkyl	:UNK0	Alkyl
:UNK0:C - A:VAL63	3.76202	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL63	Alkyl
A:PHE53 - :UNK0	4.50732	Hydrophobic	Pi-Alkyl	A:PHE53	Pi-Orbitals	:UNK0	Alkyl
A:PHE53 - :UNK0:C	4.54379	Hydrophobic	Pi-Alkyl	A:PHE53	Pi-Orbitals	:UNK0:C	Alkyl
A:TYR122 - :UNK0	5.11244	Hydrophobic	Pi-Alkyl	A:TYR122	Pi-Orbitals	:UNK0	Alkyl
A:PHE125 - :UNK0:C	4.51372	Hydrophobic	Pi-Alkyl	A:PHE125	Pi-Orbitals	:UNK0:C	Alkyl
A:PHE125 - :UNK0:C	4.18635	Hydrophobic	Pi-Alkyl	A:PHE125	Pi-Orbitals	:UNK0:C	Alkyl
A:TYR132 - :UNK0	5.32024	Hydrophobic	Pi-Alkyl	A:TYR132	Pi-Orbitals	:UNK0	Alkyl
A:TYR132 - :UNK0:C	4.33574	Hydrophobic	Pi-Alkyl	A:TYR132	Pi-Orbitals	:UNK0:C	Alkyl

9) Interaction of OBP48 (*Anopheles gambiae*) with lycopersin, azadirachtin

The OBP48 which comprised of a binding energy of -8.17, -12.05 Kcal/mol with lycopersin, azadirachtin resp. Lycopersin exhibit 6 hydrogen bond with the residues Lys21, Gly141, Arg138 and Pro22 of OBP48, azadirachtin exhibit 2 hydrogen bond with the residue Cys54 and Leu137 of OBP48. The interaction between khusimol with OBP48 obtained using LigPlot and PyMOL has been displayed in **Figure 20-21.** The detailed molecular interaction between lycopersin, khusimol and azadirachtin with OBP48 has been summarized in **Table 26-27.**

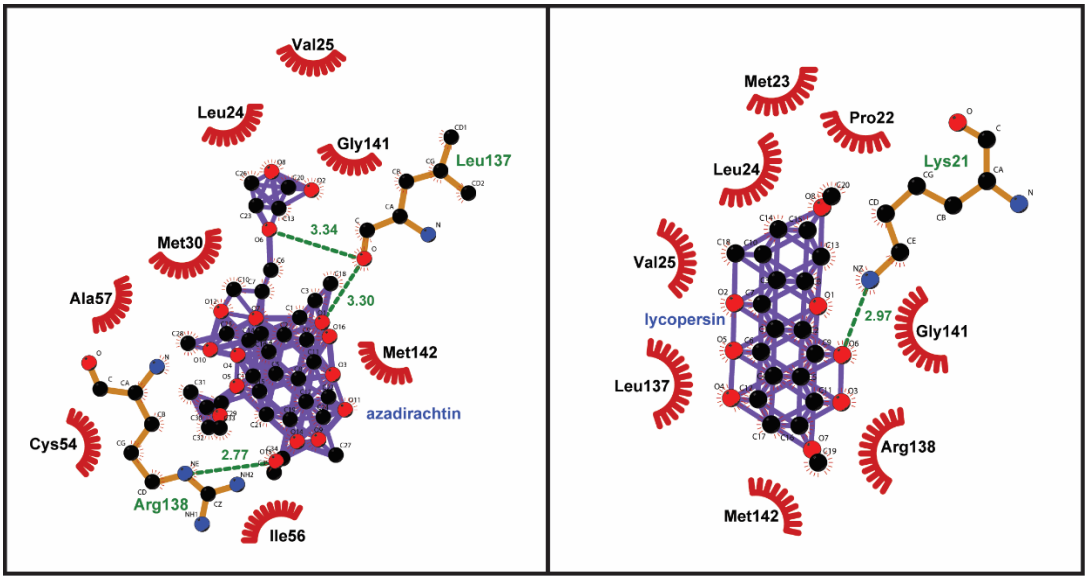

Figure 20: Molecular interaction of OBP48 with azadirachtin and lycopersin obtained using LigPlot

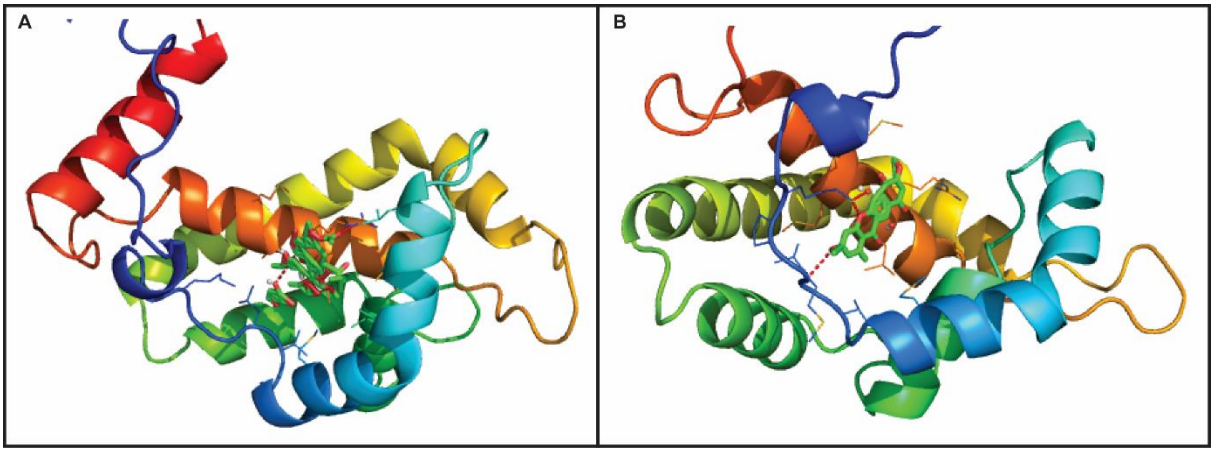

Figure 21: Molecular interaction of OBP48 with azadirachtin and lycopersin using PyMOL

Table 26: Interaction between OBP48 from *Anopheles gambiae* with lycopersin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A: LYS21:NZ - :UNK0:O	2.90516	Hydrogen Bond	Conventional Hydrogen Bond	A:LYS21:NZ	H-Donor	:UNK0:O	H-Acceptor
A:LYS21:NZ - :UNK0:O	2.97429	Hydrogen Bond	Conventional Hydrogen Bond	A:LYS21:NZ	H-Donor	:UNK0:O	H-Acceptor
A:GLY141:CA - :UNK0:O	3.52301	Hydrogen Bond	Carbon Hydrogen Bond	A:GLY141:CA	H-Donor	:UNK0:O	H-Acceptor
A:GLY141:CA - :UNK0:O	3.01951	Hydrogen Bond	Carbon Hydrogen Bond	A:GLY141:CA	H-Donor	:UNK0:O	H-Acceptor
:UNK0:C - A:ARG138:O	3.40652	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:ARG138:O	H-Acceptor
:UNK0:C - A:PRO22:O	3.09927	Hydrogen Bond	Carbon Hydrogen Bond	:UNK0:C	H-Donor	A:PRO22:O	H-Acceptor
A:LEU24 - :UNK0	4.96066	Hydrophobic	Alkyl	A:LEU24	Alkyl	:UNK0	Alkyl
:UNK0:C - A:VAL25	4.18637	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:VAL25	Alkyl
:UNK0:C - A:MET30	4.76769	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:MET30	Alkyl
:UNK0:C - A:LEU137	3.93747	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:LEU137	Alkyl

Table 27: Interaction between OBP48 from *Anopheles gambiae* with azadirachtin using Discovery Studio Visualizer

Interaction	Distance	Category	Type	From	From Chemistry	To	To Chemistry
A:ARG138:NH2 - :UNK0:O	4.53937	Electrostatic	Attractive Charge	A:ARG138:NH2	Positive	:UNK0:O	Negative
A:CYS54:SG - :UNK0:O	2.98804	Hydrogen Bond	Conventional Hydrogen Bond	A:CYS54:SG	H-Donor	:UNK0:O	H-Acceptor
:UNK0:O - A:LEU137:O	3.34321	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	A:LEU137:O	H-Acceptor
A:CYS54 - :UNK0	4.32991	Hydrophobic	Alkyl	A:CYS54	Alkyl	:UNK0	Alkyl
:UNK0 - A:ILE56	4.01873	Hydrophobic	Alkyl	:UNK0	Alkyl	A:ILE56	Alkyl
:UNK0:C - A:ILE56	4.59975	Hydrophobic	Alkyl	:UNK0:C	Alkyl	A:ILE56	Alkyl

A major challenge in combatting malaria is to develop effective yet sustainable mosquito repellents. To safeguard human health, use of safe natural compounds from plant extracts became an alternative approach to minimize the side effects as compared to synthetic mosquito repellents. Historically it is well known that plant extracts were extensively used as potential natural repellents against wide range of insects over 2000 years ago (Nentwing, 2003). Further, it has been observed that the compounds like azadirachtin, saponin, alpha-tocopherol, gamma-sitosterol, lycopersin, alpha-vetivone, beta-vetivone, gamma-selenene, khusimol, khusimone etc. from various plants like *Azadirachta indica, Ocimum tenuiflorum, Vetiveria zizanioides* exhibit strong repellent activity against malarial vectors like *Anopheles gambiae* and *Anopheles stephensi*.

In this context, *in silico* and computational simulation methods are useful for making logistic predictions and hypothesizing to find novel and effective mosquito repellents from plant based compounds. In silico molecular docking tools are quite useful in screening and identification of natural phytochemical products with insect repellent property (Venugopal and Gaddaguti, 2015; Dekker *et al*., 2011; Koech and Mwangi, 2013). These methods provide suitable insights into identifying binding residues responsible for a biological function. The present investigation was undertaken to explore the possibility of repellent activity of potential plant based natural compounds against odorant binding proteins of *Anopheles gambiae* and *Anopheles stephensi*. Further, attempts were also made to understand molecular mechanisms underlying possible interactions of natural mosquito repellent compounds against odorant binding proteins. The results from present study will open up better avenues to choose the most suitable compounds for design and development of effective and safe mosquito repellents in near future.

A total of 40 different natural repellent compounds were docked with nine odorants receptor proteins. Three compounds *i.e.*, alpha-tocopherol, gamma-sitosterol and lycopersin of *Ocimum tenuiflorum* showed strong binding affinity than DEET with most of the odorant binding proteins. In recent study Venugopal and Gaddaguti, 2015 have also revealed that compounds of *Ocimum tenuiflorum* prefers to bind OBPs with higher binding energy. These compounds from *Ocimum* plant showed strong hydrogen-bonding and hydrophobic contacts with OBPs, which is in agreement with the earlier studies (Venugopal and Gaddaguti, 2015; Dekker *et al*., 2011; and Koech and Mwangi, 2013). Similarly, few compounds like azardirachtin and saponin of *Azadirachta indica* showed storng binding affinity with OBP, OBP1, OBP4, OBP7, OBP22a and OBP48 of *Anopheles*

gambiae and OBP1 of *Anopheles stephensi*. Moreover, all compounds tested in the present study exhibit hydrogen bonding and also display hydrophobic contacts with a number of amino acids.

Previous studies have shown the activity of citronella oil and vetiver oil as mosquito repellant (Yuwadee Trongtokit, 2005). In the present study alpha-longipipene, alpha-cardinene, alpha-murolene, alpha-vetivone, beta-humulene, beta-vetivone , calacorene, calarene, gamma-selenene, khusimol, khusimone of *Vetiveria zizanioides* showed higher binding affinity with OBP, OBP1, OBP4, OBP7, OBP20, OBP22a, OBP48 of *Anopheles gambiae* and OBP1, OBP7 of *Anopheles stephensi*, as compared reference ligand DEET. Lycopersin, azadirachtin, khusimol shown to bind with more than one amino acid residues of the Odorant binding proteins by forming more than one hydrogen bonds.

Although hydrogen-bond interactions could be predicted for some binding complexes, the hydrophobic interactions had more influence on binding following hydrophobic changes that affected the cavity. The orientation of ligands affects binding by influencing hydrophobic interactions. By analyzing the molecular volume and hydrophobicity of ligands, it has been observed that the molecular volume or size in some cases fits the binding cavity and in some case it don't. In those cases when ligands with a molecular volume is large than that of the binding cavity, the ligands showed poor binding ability. This might be because of the volume of the former was so great that many collisions are induced between the cavity of the ligand, and the binding free energy is dramatically increased, which can hinder ligand binding in the pocket. Additionally, we suspect that if the ligands have a volume that is too small, it may have negative effects on binding. It is speculated that if the ligand volume is too small, the relative surface area of the interaction is also smaller and the binding ability is also poor. However, it is known that if a certain degree of conformational flexibility is presented allows the ligands to access the central binding pocket in most OBPs (Li Dong-Zhen *et al*., 2015). Further, it has been observed that protein plasticity could influence the binding range of ligands and the intensity of binding with specific ligands. In a recent study, the minus-C OBP AmelOBP14-odorant complexes have shown that the cavity volume can vary to some extent in association with ligand sizes (Ziemba BP *et al*., 2013). It could help the OBP to bind more ligands. Different levels of conformational flexibility exist in OBPs, a molecular volume that is too big could result in more collisions between atoms in the ligand and the cavity when the ligand enters the binding cavity. The importance of flexibility might reflect binding with specific ligands to enhance ligand-binding abilities. Minute observation of the ligands shows that

the ligands with greater hydrophobicity tend to have a stronger binding ability. This was also influenced by ligand orientation and steric hindrance. Thus, ligand hydrophobicity and the binding cavity had a greater influence than hydrogen bonding in various OBPs which is in agreement with earlier studies by Dong-Zhen Li *et al*., (2015).

Structure based modelling predictions may facilitate the design of novel repellents with increased binding affinity and selectivity (Tsitsanou *et al*., 2012). The monoterpenes such as camphor, carvacrol, ocimene, alpha-pinene, beta-pinene, citronellal, myrcene, geraniol, linalool etc. are constituents of essential oils showed more binding activity for most of the OBPs which have also been reported to possess mosquito repellent activity (Ibrahim *et al*., 1998; Jaenson *et al*., 2006; Park *et al*., 2005; Yang *et al*., 2004). Insect odorant binding proteins are components of olfactory system that binds to attractant and repellent odors, OBP1 protein was reported to show high binding affinity to camphor and some structurally related compounds. Therefore, the study on OBPs of *A. gambiae* and *A. stephensi*, the determination of their three-dimensional structures and binding specificities of various plant based compounds with mosquito repellent activity could help us to understand the molecular basis of odorant detection and is expected to pave the way for development of safe, effective, and environmentally friendly strategies for mosquito control.

CHAPTER V

Summary

The natural product-based products and development represents a complex endeavor demanding a highly integrated interdisciplinary approach, the presented recent scientific developments, technologic advances, and research trends clearly indicate that natural products will be among the most important sources of new drugs and mosquito repellents in the future. Earlier, the efforts to identify more effective insect repellents from plant were hindered by the absence of a known molecular target, so it was limited only to ligand-based computational approaches. With the availability of high resolution crystal structures of odorant binding proteins and high-through computational techniques, now it is possible to provide significant impetus to structure-based design of novel repellents. With more than 50 OBP-encoding genes identified so far, the list of potential molecular targets for OBP-based design of novel repellents/attractants is expected to increase substantially in the very near future. Therefore, structure-based ligand design can provide promising leads with improved binding characteristics and specificity that could be further evaluated for ORs activation. In the present study, *in silico* ligand binding of potent plant based compounds reported to possess repellent activity were screened against molecular targets of *Anopheles gambiae* and *Anopheles stephensi*. The results of our study revealed few potent compounds azadirachtin, saponin, lycopersin, alpha- vetivone, khusimol, khusimine etc. with higher binding affinity and strong hydrogen bond interaction with OBPs, which were found to be higher than that of widely used mosquito repellent DEET. The essential oils possess strong repellent activity against mosquito, insect bite and arthropod transmitted disease and is presumed to be safe as they do not cause any adverse effect to humans. The field of repellent development from the plants is extremely productive due to prosperity of insecticidal compounds found in plants as defense against insects. From our study, we have khusimol, khusimone, alpha-vetivone etc. from essential oils formed strong interaction with OBPs of both the malarial vectors. Finally, it can be concluded that the identified plant based compounds *i.e.*, with repellent activity, needs further investigation for design and development of efficient mosquito repellents than the existing harmful synthetic mosquito repellents.

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CURRICULUM VITAE

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CAREER OBJECTIVE:

To secure a challenging position in Bioinformatics environment with committed and dedicated people to contribute my Bioinformatics and Computer Skills in the field of biological research to serve our nation**.**

ACADEMIC QUALIFICATION:

Discipline	Institution	Board/University	Year of Passing	% of Marks	class
M. Sc. (Bioinformatics)	C.P.G.S,OUAT, Bhubaneswar, Orissa	Orissa University Of Agriculture & Technology	Cont.		
B.Sc. (Honours)	M.P.C (Auto.) College, Baripada, Orissa	North Orissa University, Mayurbhanj	2014	81.5%	1st
+2 Science	M.P.C Junior College, Baripada, Orissa	C.H.S.E Orissa	2011	62.16%	1st
10^{th}	Lady Hamilton Girls' High School, Baripada	H.S.E Orissa	2009	83.5%	1st

BIOINFORMATICS SKILLS:

Area of skill	Skill
Bioinformatics Tools	BLAST, FASTA, EMBOSS, Clustal W, Translate Tool Expasy, Modeller, CN3D, DISCOVERY STUDIO, MEGA,
Bioinformatics Databases	NCBI, GENBANK, SWISS-PROT, EMBL, DDBJ, PDB, SWISS-PROT

COMPUTER SKILLS:

Area Of Skill	Skill
Operating systems	MS-DOS, Windows 95/98/2000/XP, Red Hat Linux 9
Database and RDBMS	MySQL,
Programming Languages	C, Perl, HTML

AREA OF INTEREST:

Biological Database Management, Molecular Biology, Bio-chemistry, Genetics, Proteomics, Molecular Modelling, Drug Discovery & Drug Designing, Genetic Engineering.

PERSONAL INFORMATION:

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DECLARATION:

I hereby declare that the above mentioned information is correct up to my knowledge and I bear the responsibility for the correctness of the above mentioned particulars.

Place: Bhubaneswar — Swati Sucharita Satpathy

Date: — **Signature**