Molecular Interaction of Novel Phytocompounds From *Smilax Zeylanica* (L) Against the Targeted Protein of Mosquito Vectors and Their Potential Activity

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**MOLECULAR INTERACTION OF NOVEL PHYTOCOMPOUNDS FROM *SMILAX ZEYLANICA* (L)
AGAINST THE TARGETED PROTEIN OF MOSQUITO VECTORS AND THEIR POTENTIAL
ACTIVITY**

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Abstract

Malaria, dengue fever, filariasis, and viral encephalitis are all mosquito-borne illnesses that cause a great number of health issues across the world. Mosquitoes inject saliva into their hosts' skin to make obtaining a blood meal simpler by inhibiting hemostasis. D7 proteins are abundant in mosquito saliva and act as biogenic amine and eicosanoids scavengers. The chemical interactions of possible *Smilax zeylanica* compounds with mosquito-targeted D7 salivary protein and odorant binding protein were investigated. From petroleum ether, acetone, and ethanolic solvents, a total of 28 chemical components were discovered in the GC-MS study of *S. zeylanica*. Different solvents were also tested for their adulticidal and repellent properties against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. Among the three solvents, the ethanolic extract exhibited the highest toxic level against the three mosquito species. LC_{50} of P. ether 284.92ppm, 311.97ppm and 332.76ppm; LC_{50} of acetone 241.97ppm, 271.61ppm and 304.99ppm; and LC_{50} of ethanol 217.12ppm, 241.89ppm and 266.90ppm, against the three mosquito species, respectively. In repellent activity from all the solvents, complete protection was observed for up to 150 minutes against the three mosquito species. A total of 22 compounds were taken for docking studies. The compounds were subjected to docking against the three mosquitoes' D7protein. Overall, this study highlights the importance of a computational approach based on control of mosquito vectors and potential activity of biopesticides.

Keywords: D7 Protein. Mosquito Saliva. dock inhibitory compounds. Mosquito-borne illnesses. solvents

INTRODUCTION

According to the World Health Organization (WHO), mosquito-borne diseases account for about 17% of the total burden of all infectious diseases (WHO 2020a). Mosquitoes transmit a variety of diseases of medical or veterinary importance, such as malaria, filariasis, encephalitis, yellow fever, dengue, Rift Valley fever, and other diseases (Antonio-Nkondjio et al. 2019; Braack et al. 2018; Dieme et al. 2015; Parola et al. 2016; Singh et al. 2016; Tchoumbou et al. 2020; WHO, 2020b).

To understand protein-ligand interactions, molecular docking techniques are commonly utilised in the contemporary drug design process. The three-dimensional structure of the protein-ligand complex can be utilised to understand how proteins interact and conduct biological functions. As a result, one of the major difficulties in biological sciences is understanding the complete structure of protein-ligand and its complexes at the atomic level (Gaddaguti et al. 2012).

Many biological processes, including signal transduction, cell control, and other acromolecular assembly, rely on molecular recognition, which includes drug-protein interactions. Understanding the interaction mechanisms and creating therapeutic approaches require determining the binding mode and affinity between the constituent molecules in molecular recognition. Because experimental methods for establishing the structures of complexes are challenging and expensive, computational methods such as molecular docking are desired for predicting probable binding modes and affinities. (Brooijmans and Kuntz 2003; Halperin et al. 2002; Shoichet et al. 2002; Kitchen et al. 2004; Muegge and Rarey 2001; Sousa et al. 2006; Kolb et al. 2009).

The ligand-based virtual screening methods using molecular similarity searching are relatively inexpensive and widely used to pool promising molecules with comparable structures, with the probability of similar biological properties, from chemical structure databases (Ebenezer et al. 2020).

Feeding is necessary for the development of eggs, as well as the transmission of most vector-borne diseases. Because saliva and transmitted infections are so closely linked, they can be treated as a single entity, and salivary proteins are currently being tested as vaccine candidates for vector-borne diseases (Kamhawi et al. 2014; Reed et al. 2016; Pingen et al. 2017). Insects have evolved the habit of feeding on blood several times, each time resulting in the emergence of essentially novel salivary proteins (Arca et al. 2017).

Mosquito saliva especially, *Ae. aegypti* saliva contains over one hundred unique proteins that have been classified as D7 proteins, phosphatidylethanolamine binding proteins, odorant and juvenile hormone binding proteins, serpins and other protease inhibitors, a sialokinin vasodilator, nucleotidases, serine proteases, sugar digestion related proteins and other enzymes, lectins, angiopoietins, anti-microbial proteins and peptides, mucins and peritrophins, antigen 5 proteins, and many more proteins of unknown function (Chisenhall et al. 2014a,b; Bonizzoni et al. 2012).

Many researches in recent years have been noticed that the phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents, and ovipositional attractants, having deterrent activities (Rawani et al. 2010; Matasyoh 2011; Murugan et al. 2015a). Plant extracts which are being used against different stages of mosquitoes (Bilal and Hassan 2012). Applications and uses of medicinal plants products are analyzed in each level of its manufacturing processes and used by people worldwide and no side effects and cost effective compare to other system of medicine (Gangola et al. 2017).

In addition, an impressive amount of research has been published regarding the detrimental effects of pesticides, which was the main approach developed for pest control in the previous century, but which has been

progressively replaced by the use of Biological Control Agents (BCAs) and biopesticides to provide satisfactory pest management (European Commission 2021; USDA 2021).

In this study *Smilax zeylanica* is chosen for the control of mosquito and the plant is useful against skin disease, pitta, insanity, diarrhea, colic, vata syphallis, gonorrhea, fever, arthritis, leucorrhoea, impotency and general weakness etc. Till now various pharmacological activities had been done on different parts of the plant like antidiabetic, antihelmentic, antioxidant, antiepileptic, pesticidal, antigonorrheal (Hooda et al. 2011).

Hence, the present study was mainly an analysis of the phytochemical screening, and we carried out the bioactive compounds present in the different solvent leaf extracts of *S. zeylanica* using GC-MS and FTIR techniques. Also, we investigated the *S. zeylanica* leaf extract, which has been shown to have mosquitocidal properties against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. Furthermore, the molecular docking interaction mechanism of D7 salivary protein and odorant binding proteins of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* with the selected significant ligands from the plant *S. zeylanica* was also investigated.

MATERIALS AND METHODS

Collection and identification:

The fresh leaves of *Smilax zeylanica* (L) were collected in the month of November from Ooty hills, Tamilnadu, India. The plant material was taxonomically identified and authenticated by Botaniocal Survery of India, Coimbatore, Tamil nadu and the voucher specimen was retained in our laboratory for further reference (Voucher No: BSI/SRC/5/23/2017Tech./335).

Preparation of plant extract

The leaves were washed with tap water, shade-dried at room temperature ($28\pm 2^{\circ}\text{C}$) for 5 to 10 days. Since certain compounds get denatured in sunlight, it is dried under shade to avoid decomposition. 250 g of fresh, mature leaves were rinsed with distilled water and dried under shade. The dried leaves were put in a Soxhlet apparatus (Borosil Glass Workers Ltd, Mumbai, India) and extracts were prepared by Petroleum ether, acetone and ethanol [(Loba Chemie Pvt. Ltd., Mumbai, India. 99% purity) (concentration of petroleum ether, acetone and ethanol is 100%, extraction period 72 hours and the temperature was maintained $30\text{--}40^{\circ}\text{C}$)]. The yield extract was 100g and was evaporated to dryness in rotary vacuum evaporator and the dried residues obtained were stored in airtight bottles in a refrigerator for further use.

Preliminary phytochemical analysis

The leaf petroleum ether, acetone and ethanol of *S. zeylanica* was subjected to phytochemical analysis was carried out for identification of tannins, terpenoids, flavonoid, alkaloid, phenol and steroids according to standard methods (Sofowara 1993; Harborne 1973; Ogbuewu 2008) is described in **Table 1**.

Gas chromatography-mass spectrum (GC-MS) analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, $30\text{ m} \times 0.25\text{ mm ID} \times 250\mu\text{m df}$) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1 μL of extract sample injected into the instrument the oven temperature was as

follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da.

Identification of Components

The Identification of compounds was done based on the retention indices, molecular structure, molecular mass spectra and calculated fragmentation patterns with those stored on the computer library and also with published literature. Interpretation on mass spectrum GC MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library and WILEY9 (Van Den Dool and Kratz 1963) on-line library source was also used for matching the identified components.

Fourier transforms infrared spectrophotometer (FTIR) analysis:

The *S. zeylanica* leaf extract of petroleum ether, acetone and ethanol were prepared by dried powder used for the FTIR analysis. 10mg of the dried leaf extract powder was condensed in 100 mg of KBr pellet after the each sample was loaded in the infrared spectra of the solid samples in the range 400 to 4000 cm⁻¹ were recorded on JASCO-4100 models spectrophotometer using KBr pellets.

Structural and Biological Data

Publicly available structural and biological data for proteins and ligands were used throughout this work. Online bioinformatics databases such as Pub Med, PDB, Pub Chem and Drug Bank were used.

Databases Accessed

Protein Data Bank

The RCSB PDB is freely available in the public domain. It is hosted and managed by the Research Collaboratory for Structural Bioinformatics (RCSB). It can be accessed at <http://www.pdb.org/pdb/home/home.do>. Currently 151579 (as on 06 May, 2019) structures are available in PDB. The three dimensional structure of the template was obtained from Protein Data Bank.

PubChem

It can be accessed freely at <http://pubchem.ncbi.nlm.nih.gov/>.

Retrieval of compounds and ligand preparation

Build panel

The 3Dmodel generated was viewed with the help of the visualization tool Pymol (<http://www.pymol.org>).

Ligand preparation

Ligand preparation used by LigPrep tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in sdf or Maestro format.

Protein selection

Three Dimensional structures (3D) of the target proteins, namely *Aedes aegypti* D7 & Odorant protein (PDB ID: 3DXL, 3K1E), *Anopheles stephensi* D7 protein (PDBi ID: 3NGV) and *Culex quinquefasciatus* D7 & Odorant protein (PDB ID: 3DXL, 2L2C) were retrieved from Protein Data Bank (www.rcsb.org).

Schrodinger

Schrödinger software suite is drug designs of software using both ligand and structure-based methods. It provides superior solutions and services for the design, selection and optimization of novel drug candidates.

Protein preparation

The entire procedure can be performed in the Protein Preparation Wizard panel (Schrödinger software suite), from the Workflows menu on the main toolbar. The intended workflow in this panel is from top to bottom: fixing structures first, and then deleting unwanted chains and waters, then fixing or deleting het groups, and finally performing some optimization of the fixed structure. Glide calculations use an all-atom force for accurate energy evaluation.

Receptor grid generation

Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. The options in each tab of the Receptor Grid Generation panel allows to define the receptor structure by excluding any co-crystallized ligand that may be present, determine the position and size of the active site as it will be represented by receptor grids, and set up Glide constraints.

Ligand docking jobs cannot be performed until the receptor grids have been generated. Receptor grid generation requires a “prepared” structure: an all atom structure with appropriate bond orders and formal charges. The Receptor Grid Generation panel has three tabs, which is used to specify settings for the receptor grid generation job:

- Receptor
- Site
- Constraints

Molecular docking

Grid files represent active site of the protein that is searched when attempting to dock inhibitory compounds. All the prepared inhibitory compounds were docked against to the grid generated target proteins. Molecular docking was performed using Glide module from Schrodinger suite.

The inhibitory compounds used for docking, it was initially screened using Standard Precision (SP) docking followed by Extra Precision (XP) docking. The XP method weeds out false positives and provides better correlation between good poses and good scores. Since XP mode is a refinement tool designed for use

only one inhibitory compounds poses, large sets of compounds are screened by the SP mode. Ultimately, optimization of the compound structures in the field of the receptor was done and the compounds were scored. Glide was run in rigid mode; the latter automatically generates conformations for each inhibitory compounds. The combination of position and orientation of inhibitory compounds relative to the target protein receptor, along with its conformation is referred to as an inhibitory compounds pose. The inhibitory compounds poses that Glide generates pass through a series of hierarchical filters that evaluate the inhibitory compounds interaction with the target proteins. The initial filters validate the spatial fit of the inhibitory compounds to the defined active site of the target proteins. Final scoring is then carried out on the energy-minimized poses. Scoring function is used to score the poses. Glide Score was selected as the scoring function to rank the poses of each inhibitory compound. Finally, Glide XP Visualizer panel is used to Visualizing the docking complex.

Mosquito Rearing

Experiments were conducted using laboratory-reared pathogen-free strain strains of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. Colonies were originally established as described by Dinesh et al. (2015) respectively. Eggs of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were provided by National Centre for Disease Control (NCDC), field station of Mettupalayam, Coimbatore (Tamil Nadu, India) using an “O” type brush. These eggs were brought to the laboratory and were transferred to 18 X 13 X 4 cm size enamel trays containing 500 ml of water and kept for larval hatching. Mosquito larvae were maintained at 27±2°C, 75–85% R.H.; (relative humidity), under a photoperiod of 14:10 (light/dark). Mosquito larvae were fed daily with 5 g of ground dog biscuits (Pedigree, USA) and hydrolyzed yeast (Sigma-Aldrich, Germany) in a 3:1 ratio. Newly emerged larvae and pupae and 2-day-old adults were collected and used in the experiments.

Adulticidal bioassay

Adulticidal experiments were performed following the methods reported by Suresh et al. (2015). The leaf extract of *S. zeylanica* was tested at 100, 200, 300, 400, and 500 ppm. For each tested dosage, five replicates were carried out. The leaf extract were applied on Whatman no. 1 filter paper (size 12 × 15 cm) lining a glass holding tube (diameter 30 mm; length 60 mm). In control treatments, filter paper was treated with either the same volume of distilled water or in aqueous solution. In each test, 20 mosquito females were gently transferred into another glass holding tube. The mosquitoes were allowed to acclimatize in the tube for 1 h and then exposed to a test tube lined with treated or control paper for 1 h. At the end of exposure period, the mosquitoes were transferred back to the original holding tube, kept for a 24-h recovery period, then mortality was recorded. A pad of cotton soaked with 10 % (w:v) glucose solution was placed on the Sugar-fed adult female mosquitoes (5 to 6 days old) were used. The biopesticides were diluted with acetone to make different concentrations. The diluted biopesticides were impregnated on filter papers (140×120 mm). A blank paper consisting of only ethanol was used as control. The papers were left to dry at room temperature to let the ethanol evaporate overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring 125×44 mm following the method of WHO (1981). One tube served to expose the mosquitoes to the biopesticides and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 16-mesh wire screen. Sucrose-fed and blood-starved mosquitoes (20) were released into the tube, and the mortality effects of the bio pesticides were observed every 10min for a 3hrs

exposure period. At the end of 1, 2 and 3 hrs exposure periods, the mosquitoes were placed in the holding tube. Cotton pads soaked in 10% sugar solution with vitamin B complex was placed in the tube during the holding period for 24 hrs. Mortality of the mosquitoes was recorded after 24 hrs. The above procedure was carried out in triplicate for bio pesticides of each concentration. The lethal concentrations (lethal dose LC₅₀, LC₉₀) were calculated by probit analysis (Finney 1971).

Repellent activity

The repellency of the *S. zeylanica* extracts against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* was evaluated by using the percentage of protection in relation dose method. Three days old blood starved female *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes (100) were kept in to net cage (45cm×30cm×45cm). Two cages with hungry mosquitoes for test and control were kept aside. The volunteer had no contact with lotions, perfumes, oils or perfumed soap on the day of assay. The arms of the volunteer skin washed and cleaned with ethanol and ethanol served as control. After air drying, the arms of the volunteer, only 25 cm² dorsal side of the skin on each arm was exposed and the remaining area covered by rubber gloves. The different concentrations of crude extracts were applied. *Ae. aegypti* was tested during the day time from 07.00 to 17.00 h, while *Cx. quinquefasciatus* and *An. stephensi* were tested during the night from 19.00 to 05.00 h. The control and treated arm were introduced simultaneously into the mosquito cage, and gently tapping the side on the experimental cage, the mosquitoes were activated. The volunteer conducted their test of each concentration by inserting the treated and control arm into cages at a same time for one full minute for every 5 min. The experiment was trialed 5 times. The mosquitoes that land on the hand were recorded and then shaken off before imbibes any blood. The percentage of repellency was calculated by formula.

$$\text{Protection} = \frac{(\{\text{No. of bites received by control arm}\} - \{\text{No. of bites received by treated arm}\}) \times 100}{\text{No. of bites received by control arm}}$$

Data analysis

The average mosquito larvicidal, pupicidal and adulticidal toxicity data were subjected to probit analysis. LC₅₀ and LC₉₀ were calculated using the method by Finney (1971). All data were analyzed using the SPSS Statistical Software Package version 16.0. The mosquito ovicidal, larvicidal and adulticidal percentage data were analyzed also subjected to ANOVA followed by Tukey's HSD test. A probability level of P<0.05 was used for the significance of differences between values.

RESULTS AND DISCUSSION

Primary phytochemicals screening of *Smilax zeylanica*

Tannins: A green precipitate was observed in all the extracts indicating thereby the presence of tannins in all three solvent extracts analysed.

Terpenoids: A deep red color was observed in ethanol extracts out of three solvents.

Flavonoids: A yellow coloration was also observed in all the solvent extracts indicating thereby the presence of flavonoids in all three solvent extracts screened.

Alkaloids: A yellow precipitate was observed in two solvent extracts except petroleum ether extract and confirming thereby the presence of alkaloids.

Phenolic: An intense colour was observed in all solvent extracts and confirming thereby the presence of phenolic.

Steroids: A reddish brown ring at the interface was observed in all the three solvent extract of *S. zeylanica* and indicating the presence of steroids in this plant. **(Given in table 2)**

S. zeylanica is contained chemicals such as diosgenin, smilagenin, sarsasapogenin, β -sitosterol, hydroxytyrosol, trans-iso-eugenol and squalene (Murali et al. 2012; Rajesh and Perumal 2014). Recently, Padma et al. (2019) reported that the phytochemical characterization, ethanolic leaves extracts of *S. marianum* revealed the presence of alkaloids, flavonoids, tannins, phenol, cardiac glycosides, anthraquinones and also revealed the absence of saponins, terpenoids. The results agree with the finding of Dhawan and Gupta et al. (2017) observed the different phyto-constituents such as alkaloids, flavonoids, saponins, steroids and tannins were present in the acetone, chloroform, distilled water, ethyl acetate, hexane and methanol extract of *Datura metel*. Pandey et al. (2017) noted that the following phytochemicals such as alkaloid, saponin, protein, quinone, sterols, cardiac glycoside, flavonoids, tannin, terpenoid, reducing sugar in the methanol extract of *A. vulgaris* and *G. fragrantissima*.

GC-MS analysis

In the present study, GC-MS chromatogram was detected in the following three solvent (petroleum ether, acetone and ethanol) leaf extracts of *S. zeylanica* **(illustrated in fig. 1-3)**. The identification of the phytochemical compounds present in the various solvent extract of *S. zeylanica* was confirmed based on their retention time (RT), molecular formula, molecular weight, and percentage (% area), Forward, Reverse and biological properties are given in **Table 3 to 5**.

The GC-MS analysis of petroleum ether leaf extracts of *S. zeylanica* revealed the presence of 10 different bioactive compounds namely, Phytol; 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol, N-Hexadecanoic Acid, Pentadecanoic Acid, 1-Hexyl-2-Nitrocyclohexane, E-3-Pentadecen-2-ol, Hexadecane 1-Chloro-, Tritetracontane, Heptacosane, 1-Chloro and Vitamin E respectively, are shown in **(Table 3)**. The acetone leaf extract of *S. zeylanica* showed the presence of 8 different bioactive compounds namely, 2-(2-Piperidin-1-yl-Ethyl)-Pyridine, 3-Acetoxydodecane, N-Hexadecanoic Acid, Hexadecanoic Acid, 2-Bromo-, Pentanoic Acid, 2-(Aminoxy)-, Bicyclo[3.2.1]oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-Methoxy-1,3-, 2-Isopropyl-5-Methylcyclohexyl-3-(1-(4-Chlorophenyl)-3-Oxobutyl)-C, 1-Monolinoleoylglycerol Trimethylsilyl Ether respectively Presented in the **Table 4**. Ethanol extract of *S. zeylanica* contains 10 different secondary compounds namely, 2-Pentenal, 2-Methyl, 4,4'-Biscyclohexanone, 2,2',6,6'-Tetramethyl-, 6-Methyl-7-oxo-1,7-Dihydro-Triazolo (4,3-B) Triazine, 6-Acetyl-.Beta.-D-Mannose, 4-Ethyl-2-Hydroxycyclopent-2-en-1-one, Cyclopropanetetradecanoic Acid, 2-Octyl-, Methyl Ester, D-Galactitol, 1,3,4,5-Tetra-O-Methyl-, Diacetate, Eicosanoic Acid, 2,3-bis[(Trimethylsilyl)oxy]Propyl Ester, 11-Eicosenoic Acid, Trimethylsilyl Ester, Ethanol, 1-(Methylenecyclopropyl)-1-(Methylene-1-Trimethylsilyl) respectively, were shown in **(Table 5)**.

We observed that GC-MS analysis indicated the ethanol extract had present in the maximum bioactive compounds where as compared with other solvent extract of petroleum ether followed by acetone extract respectively. **Table 6** Showed the biological properties listed in the presence of various phytochemical compounds from petroleum ether, acetone and ethanol leaf extract of *S. zeylanica*.

The FTIR spectrum analysis using for the identification of major peaks correspondence to the functional groups and bioactive active components were present in the petroleum ether, acetone and ethanol leaf extract of *S. zeylanica*. In the present results, FT-IR analysis observed the peak at 3352, 2962, 1707, 1366, 1229, 1027 and 681 cm^{-1} which correspond to confirmed the presence of alcohol, Amine group, Carbocyclic acids, Phenol, Ether, Alkyl aryl ether and Alkene (**Table 7 and Fig. 4**). Kalaichelvi and Dhivya (2017) reported that FT-IR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, aromatics, nitro compounds and amines in petroleum ether extract of *Micrococca mercurialis*. Starlin et al. (2012) reported the ethanolic extract of *Tylophora pauciflora* observed the peak at 2800, 2862.36 and 2926.01 cm^{-1} which correspond to lipids, alkanes, and hydroxyl compounds and the peak at 1730, and 1708 cm^{-1} shows the presence of ester carbonyl and unsaturated carbonyl groups. The strong absorption bands at 2931 and 1458 cm^{-1} are due to CH and CH_2 groups respectively.

Molecular interaction studies of inhibitory compounds against target proteins

One key methodology ideated during 1980 of docking of small molecules to protein binding sites remains a highly active area of research especially in conditions when the structure of a target and its active or binding site is available, therefore, primarily it was used as a hit identification tool. Further validated compounds used for wet lab practice. Specific features of protein-ligand docking methods and several applications were used here in combination with hit identification and lead optimization (Bajorath 2015).

The compounds taken from the GCMS studies and retrieved from the pubchem database and used for docking studies. The list of compounds retrieved is providing in the (**Table 8**). Totally 23 screened compounds were subjected for docking against *Ae. Aegypti* D7 protein and *Cx. quinquefasciatus* (PDB ID: 3DXL). Initially, based on scoring function, all compounds were screened. Glide score of 6-Acetyl-.Beta.-D-Mannose was -8.75. 6-Acetyl-.Beta.-D-Mannose compound have good glide score and also potentially interacted with D7 (**Fig. 5**). Out of 23 compounds 6-Acetyl-.Beta.-D-Mannose docking score was -8.75 that formed 8 hydrogen bonds interacting with GLU18, GLU18, ARG270, ARG15, THR14, ARG267, ARG267, ARG267 residue and Top ranking compounds showed good glide score (**Table 9**).

Similarly, docking of potential inhibitory compounds against *Anopheles stephensi* D7 protein (PDB ID: 3NGV). Glide score of Bicyclo[3.2.1]oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-Methoxy-1,3 was -7.24 and one H bond formed in between H atom of Bicyclo[3.2.1]oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-Methoxy-1,3. ARG199 and CYS55 residue of D7 protein (**Fig. 6**). Top ranking compounds showed good glide score and also more number of H-bonds (**Table 10**).

Docking of potential inhibitory compounds against *Aedes aegypti* Odorant protein (PDB ID: 3K1E). Glide score of Bicyclo[3.2.1]oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-Methoxy-1,3 was -10.89 and one H bond formed in between H atom of Bicyclo[3.2.1]oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-Methoxy-1,3 and HIE111 residue of D7 protein (**Fig. 7**). Top ranking compounds showed good glide score and also more number of H-bonds (**Table 11**).

Docking of potential inhibitory compounds against *Culex quinquefasciatus* Odorant protein (PDB ID: 2L2C). Glide score of Bicyclo [3.2.1] oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-Methoxy-1,3 was -10.45 (**Fig. 8**). Here, as per scoring and interaction, top 5 compounds were selected which showed a score range of -

7.82 to -10.45. Top ranking compounds showed good glide score and also more number of H-bonds given in (Table 12).

However D7 proteins are directly responsible for insecticide resistance or simply closely-associated markers, their role in mosquito blood-feeding makes this over expression phenotype relevant to malaria transmission. Real-time quantitative PCR analyses of mosquitoes collected post-IRS detected instances of extremely high levels of D7r2 and D7r4 expression. D7 proteins are estimated to compose at least 5–20% of salivary protein Calvo et al. (2006).

Our results compared with recently studied that the D7 protein from the *Aedes aegypti* mosquito was observed to bind dengue virions and envelope protein, suggesting that this family of proteins may have a broader range of associated phenotypes Conway et al. (2016). In addition Isaacs et al. (2018) investigated the *insilico* docking studies confirmed that bendiocarb could be manually positioned in the D7r4 salivary protein against *An. gambiae*. Although D7r2 and D7r4 are relatively distantly related, sharing only 32% sequence identity, they align with only a single one residue insertion in the former vs the latter.

Further present findings agree with those earlier studies of Carey et al. (2010) reported that the some plant-derived repellents have been found to target multiple odorant receptors. Expected to be less susceptible to resistance than compounds acting on a single receptor (Norris and Coats 2017). Thireou et al. (2018) studied a fast and efficient virtual screening protocol for discovery of novel bioinspired synthetic mosquito repellents with lower volatility and, in all likelihood, increased protection time as compared with their plant-derived parental compounds.

Adulticidal activity

Adulticidal activity of p.ether, acetone and ethanol leaf extract of *S. zeylanica* against adult of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. among the leaf extract of *S. zeylanica* was found to cause a mosquito knockdown after exposure to various concentrations paralysis and inactive. The results of the adulticidal activity of p.ether leaf extract of *S. zeylanica* against adults of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* led to LC₅₀ and LC₉₀ value of *An. Stephensi* 284.92 (678.15) ppm; *Ae. aegypti* 311.97 (700.05)ppm; *Cx. quinquefasciatus* 332.76 (715.34ppm), respectively (given in table 13).

Table 14 provides adult mortality of acetone extract of *S. zeylanica*, showed *An. stephensi*, as measured by LC₅₀ and LC₉₀ values of 241.97 and 634.40ppm; *Ae. aegypti* LC₅₀ value was 271.61ppm and LC₉₀ value was 662.33ppm; *Cx. quinquefasciatus* LC₅₀ value was 304.99ppm and LC₉₀ value was 703.03ppm, respectively. The subsequent record at the end of a 24 hrs holding period revealed mortality values of, p.ether, acetone, and ethanol was 79.2%, 76.4%, and 72.3%, at 500 ppm, respectively.

Table 15 shows the ethanol leaf extracts of *S. zeylanica* showed statistically significant toxicity on the adult of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The mortality rates were directly proportional to concentration of the *S. zeylanica* leaf extracts used. The highest adulticidal activity was observed in the *An. stephensi* followed by, *Ae. aegypti*, and *Cx. quinquefasciatus*. Th *An. stephensi*, as measured by LC₅₀ and LC₉₀ values of 217.12 and 578.86ppm; *Ae. aegypti* LC₅₀ value was 241.89ppm and LC₉₀ value was 607.15ppm; *Cx. quinquefasciatus* LC₅₀ value was 266.90ppm and LC₉₀ value was 642.43ppm, respectively.

Present findings also agree with those from earlier studies of Amerasan et al. (2012) who have reported that the all extracts showed moderate adulticidal effects; however, the highest adult mortality observed was found in methanol extract. The LC₅₀ and LC₉₀ values of *C. tora* leaf extract against adulticidal activity of

(hexane, chloroform benzene, acetone, and methanol) *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* were the following: *Cx. quinquefasciatus* LC₅₀ values were 338.81, 315.73, 296.13, 279.23, and 261.03 ppm and LC₉₀ values were 575.77, 539.31, 513.99, 497.06, and 476.03 ppm; *Ae. aegypti* LC₅₀ values were 329.82, 307.31, 287.15, 269.57, and 252.03 ppm and LC₉₀ values were 563.24, 528.33, 496.92, 477.61, and 448.05 ppm; and *A. stephensi* LC₅₀ values were 317.28, 300.30, 277.51, 263.35, and 251.43 ppm and LC₉₀ values were 538.22, 512.90, 483.78, 461.08, and 430.70 ppm, respectively. Pratheeba et al. (2015) reported that the adulticidal activities of acetone, hexane and chloroform extracts of *Ocimum gratissimum* against filariasis vector *Cx. quinquefasciatus*.

Repellent activity of *S. zeylanica* leaf extracts against *An. Stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*

In the present study showed that repellent of *S. zeylanica* origin do not pose hazards of toxicity to human and domestic animals and are easily bio-degradable. Natural products are safe for human when compare with synthetic compounds certain natural products have been investigated for repellent against mosquitoes. The present investigation results suggest that among the three solvent extracts such as p.ether, acetone and ethanol leaf extract of *S. zeylanica* exhibited better protection against the bite from *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The ethanol had the maximum protection followed by acetone and p.ether leaf extract of *S. zeylanica* against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* respectively. The skin repellent activity of petroleum ether, acetone and ethanol leaf extract of *S. zeylanica* against *An. Stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. When the repellent activity of three different concentrations were tested (0.5, 1.0, 1.5mg/cm²) on volunteer skin (forearm). The result revealed that the protection observed at 1.5mg/cm² was more protection than the other two concentrations. Among the tested ethanol extract provided significant repellency against *An. Stephensi* followed by *Ae. aegypti* and *Cx. quinquefasciatus* respectively. The higher concentration of 1.5mg/cm² provided 100% protection up to 210 minutes. The lower concentration of 0.5 mg/cm² and 1.0mg/cm² provided 100% protection up to 180 minutes, respectively (**Table:16-18**).

Repellent is known to play important role in preventing the vector borne disease by reducing man-vector contact. Synthetic chemicals and insecticides used for control of vectors are causing irreversible damage to the eco-system, as some of them are non-degradable in nature. The plant based repellents may have good role in reducing the biting nuisance and infections by preventing the man-fly contact (Fradin and Day 2002; Spero et al. 2008) and to preclude any adverse effect that could emanate from the use of synthetic repellents (Goodyer and Behrens 1998).

Our results agree with some previous studies, Hassan et al. (2015) reported that the methanol, acetone and petroleum ether extracts of *Ocimum basilicum* and *Glycyrrhiza glabra* were screened for their repellency effect against *Cx. pipiens*. Murugan et al. (2012) Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Al-Sarar et al. (2014) evaluated that the *Mentha longifolia* L, and *Lavandula dentata* L essential oils had excellent insecticidal and repellent activity against adult females of *Culex pipiens* L. Further, Sritabutra and Soonwera (2013) reported that repellent activity of herbal essential oils against *Aedes aegypti* and *Culex quinquefasciatus*.

Lastly, Govindarajan et al. (2011) studied the repellent activities of crude benzene and ethyl acetate extracts of leaf of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were assayed for their toxicity against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

Conclusion

In this study, we have determined the compound present in *S. zeylanica* was screened for their mosquitoes by docking them with D7 and Odorant Binding proteins. identified and characterised binding sites of target odorant binding proteins (OBPs) D7 and from *An. stephensi*, *A. aegypti*, and *Cx. quinquefasciatus*. The molecular docking study clearly states that the D7 protein and plant components had excellent interactions between them. Furthermore, It was investigated whether these compounds could be used as adulticidal and repellents against the three mosquito species, and significant results were obtained. Overall, the study concluded that as the identified ligands are of phytochemical origin, it may be presumed that they are safer in the environment as well as less toxic to targeted organisms.

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Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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FIGURES

Molecular interaction of Novel Phytocompounds from *Smilax zeylanica* (L) against the targeted protein of mosquito vectors and their potential activity

Journal: Environmental Science and Pollution Research

FIGURE – 1.

Fig. 1: GC-MS chromatogram of the petroleum ether leaf extract of *Smilax zeylanica*

FIGURE – 2.

Description:

GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP

Sample ID: ACE-(17ES-0547)

Page 1 of 1

Vial Number: 24

Fig. 2: GC-MS chromatogram of the acetone leaf extract of *Smilax zeylanica*

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FIGURE – 3.

Description:

GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP

Sample ID: ET-(17ES-0548)

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Vial Number: 25

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768 **Fig. 3:** GC-MS chromatogram of the ethanol leaf extract of *Smilax zeylanica*

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FIGURE – 4.

Fig. 4: FTIR analysis leaf extract of *Smilax zeylanica*

FIGURE – 5.

Fig. 5: Docked Complex of 6-Acetyl-.Beta.-D-Mannose with *Aedes aegypti* and *Culex quinquefasciatus* D7 protein (PDB ID : 3DXL)

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FIGURE – 6.

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826 **Fig. 6:** Docked Complex of Bicyclo[3.2.1]oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-
827 Methoxy-1,3 with *Anopheles stephensi* D7 protein (PDB ID : 3NGV)

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FIGURE – 7.

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845 **Fig. 7:** Docked Complex of Bicyclo [3.2.1] oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-
846 Methoxy-1,3 with *Aedes aegypti* Odorant protein (PDB ID : 3K1E)

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FIGURE – 8.

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865 **Fig. 8:** Docked Complex of Bicyclo[3.2.1]oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-
866 Methoxy-1,3 with *Culex quinquefasciatus* Odorant protein (PDB ID : 2L2C)

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TABLES

Table 1: Preliminary phytochemical tests for plant extracts

Phytoconstituents	Test	Observation
Tannins (Braymer's Test)	2ml extract + 2ml H ₂ O + 2-3 drops FeCl ₃ (5%)	Green precipitate
Terpenoids	2ml extract + 2ml (CH ₃ CO) ₂ O + 2-3 drops conc. H ₂ SO ₄	Deep red coloration
Flavonoids	1ml extract + 1ml Pb(OAc) ₄ (10%)	Yellow coloration
Alkaloids (Hager's Test)	2ml extract + few drops of Hager's reagent	Yellow precipitate
Phenolic	2ml extract + 2ml H ₂ O + 0.5ml of FeCl ₃ (w/v)	formation of an intense colour
Steroids (Salkowski Test)	(2ml extract + 2ml CHCl ₃ + 2ml H ₂ SO ₄ (conc.)	Reddish brown ring at the junction

891 **Table 2:** The primary phytochemicals screening leaf extract of *Smilax zeylanica*

Sl.No.	Phytochemical	P. ether	Acetone	Ethanol
1	Tannins	-	+	+
2	Terpenoids	-	-	+
3	Flavonoids	+	+	+
4	Alkaloids	-	+	+
5	Phenolic	+	+	+
6	Steroids	+	+	+

892 + = Present and - = Absent

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903 **Table 3:** GC-MS profiles revealed the phytochemical compounds presence in the petroleum ether leaf extract of *Smilax zeylanica*

Sl. No	RT	Name of the compounds	Structure and MF	MW	Area (%)	For	Rev
1	18.910	Phytol	C₂₀H₄₀O	296	2.426	880	936
2	19.295	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	C₂₀H₄₀O	296	4.375	789	894
3	20.776, 21.026	N-Hexadecanoic Acid	C₁₆H₃₂O₂	256	10.985,1.983	860, 826	934,915
4	21.171, 21.426, 21.926	Pentadecanoic Acid	C₁₅H₃₀O₂	242	3.574, 3.030, 24.734	838, 822, 891	896, 902, 932
5	22.506, 23.382	1-Hexyl-2-Nitrocyclohexane	C₁₂H₂₃O₂N	213	17.997,8.629	483,449	873, 846
6	24.157	E-3-Pentadecen-2-ol	C₁₅H₃₀O	226	4.318	626	866
7	24.792	Hexadecane, 1-Chloro-	C₁₆H₃₃Cl	260	1.623	555	857
8	26.013	Tritetracontane	C₄₃H₈₈	604	5.017	801	941
9	27.288	Heptacosane, 1-Chloro-	C₂₇H₅₅Cl	414	6.869	768	919
10	28.063	Vitamin E	C₂₉H₅₀O₂	430	4.442	435	653

904 RT- Retention time; P- Probability; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse.

905 **Table 4:** GC-MS profiles revealed the phytochemical compounds presence in the acetone leaf extract of *Smilax zeylanica*

Sl. No	RT	Name of the compounds	MF	MW	Area (%)	For	Rev
1	11.582, 11.637, 11.822	2-(2-Piperidin-1-yl-Ethyl)-Pyridine	$C_{11}H_{17}N_3$	191	16.148, 15.996, 28.687	358, 367, 359	854, 839, 845
2	20.535	3-Acetoxydodecane	$C_{14}H_{28}O_2$	228	7.133	489	706
3	22.546	N-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256	6.572	468	770
4	22.891	Hexadecanoic Acid, 2-Bromo-	$C_{16}H_{31}O_2Br$	334	9.040	355	675

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5	23.317	Pentanoic Acid, 2-(Aminooxy)-	$\text{C}_5\text{H}_{11}\text{O}_3\text{N}$	133	2.844	335	805
6	23.857, 24.642	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-Methoxy-1,3-	$\text{C}_{21}\text{H}_{24}\text{O}_7$	388	3.134, 2.575	387, 321	758, 702
7	23.902	2-Isopropyl-5-Methylcyclohexyl 3-(1-(4-Chlorophenyl)-3 Oxobutyl)-C	$\text{C}_{30}\text{H}_{33}\text{O}_6\text{Cl}$	524	5.849	314	883
8	27.018	1-Monolinoleoylglycerol Trimethylsilyl Ether	$\text{C}_{27}\text{H}_{54}\text{O}_4\text{Si}_2$	498	2.021	399	697

907 RT- Retention time; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse.

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912 **Table 5:** GC-MS profiles revealed the phytochemical compounds presence in the ethanol leaf extract of *Smilax zeylanica*

Sl. No	RT	Name of the compounds	MF	MW	Area (%)	For	Rev
1	11.547	2-Pentenal, 2-Methyl-	$C_6H_{10}O$	98	19.699	421	802
2	15.493	4,4'-Biscyclohexanone, 2,2',6,6'-Tetramethyl-	$C_{16}H_{26}O_2$	250	11.832	512	772
3	16.434	6-Methyl-7-oxo-1,7-Dihydro-Triazolo(4,3-B)Triazine	$C_5H_5ON_5$	151	3.303	337	703
4	17.139	6-Acetyl-.Beta.-D-Mannose	$C_8H_{14}O_7$	222	5.571	455	680
5	17.734	4-Ethyl-2-Hydroxycyclopent-2-en-1-one	$C_7H_{10}O_2$	126	6.809	383	888

6	19.260	Cyclopropanetetradecanoic Acid, 2-Octyl-, Methyl Ester	$C_{26}H_{50}O_2$	394	8.344	478	714
7	20.245	D-Galactitol, 1,3,4,5-Tetra-O-Methyl-, Diacetate	$C_{14}H_{26}O_8$	322	5.267	319	631
8	20.485	Eicosanoic Acid, 2,3-bis[(Trimethylsilyl)oxy]Propyl Ester	$C_{29}H_{62}O_4Si_2$	530	12.522	372	676
9	21.996	11-Eicosenoic Acid, Trimethylsilyl Ester	$C_{23}H_{46}O_2Si$	382	10.744	384	718
10	23.091	NV	--	--	12.262	--	--
11	25.878	Ethanol, 1-(Methylenecyclopropyl)-1-(Methylene-1-Trimethylsilylcy	$C_{13}H_{22}OSi$	222	3.647	348	617

913 RT- Retention time; NV- Not validated; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse.

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915 **Table 6:** GC-MS analysis showed the phytochemical compounds presence in the leaf extract of *Smilax zeylanica* and their biological activities

S. No	Name of the compounds	Biological properties	References
1.	Phytol	Mycobacteria, anticonvulsant, antispasmodic, anticancer activities, Precursor for manufacture of Vitamin E, antimicrobial, anti-diuretic, anti-inflammatory, immuno stimulatory and anti-diabetic activity	(Saikia <i>et al.</i> , 2010; Costa <i>et al.</i> , 2012; Sermakkani <i>et al.</i> , 2012)
2.	3,7,11,15Tetramethyl-2-Hexadecen-1-ol	Terpene alcohol and anti-inflammatory, antimicrobial, flavoring agent, cancer-preventive, anti-diuretic, antioxidant	(Jegadeeswari, <i>et al.</i> , 2012; Shibula <i>et al.</i> , 2015)
3.	N-Hexadecanoic Acid	Anti-inflammatory, Antioxidant, hypocholesterolemic nematocide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor, potent mosquito larvicide,antioxidant, hypocholesterolemic, nematocide, pesticide anti-androgenic, flavor, hemolytic, 5 -alpha -reductase inhibitor, potent mosquito larvicide	(Dandekar <i>et al.</i> , 2015; Soosairaj and Dons 2016)
4.	1-Hexyl-2-Nitrocyclohexane	Neuroactive, anti-inflammatory, analgesic Property	Dinesh <i>et al.</i> (2014)
5.	Tritetracontane	Anti-inflammatory	Isaiah <i>et al.</i> (2016)
6.	Vitamin E	Lipid and antiaging, anti - Alzheimer, antidermatitic,antidiabetic, antioxidant, antitumor, cancerpreventive,hypocholesterolemic, immunostimulant, antiaging, analgesic, antidiabetic, anti - inflammatory, antioxidant, antidermatitic,antileukemic, antitumor, anticancer, hepatoprotective,hypocholesterolemic, antiulcerogenic, vasodilator,antispasmodic, antibronchitic, anticoronary	(Shahina <i>et al.</i> 2016; Kumaravel <i>et al.</i> , 2017)
7.	3-Acetoxydodecane	No activity	Karthik Prabu <i>et al.</i> (2013)
8.	PentanoicAcid,2-Aminooxy)-	Antimicrobial	Duke (2000)
9.	2-Isopropyl-5-Methylcyclohexyl 3-(1-(4-Chlorophenyl)-3-Oxobutyl)-Coumarin-4-	Antioxidant, antimicrobial and anti-inflammatory activity	Sahoo <i>et al.</i> (2012)

	YlCarbonate		
10.	1-Monolinoleoylglycerol Trimethylsilyl Ether	Antimicrobial, Antioxidant, Antiinflammatory, Diuretic, Antiarthritic, Antiasthma	Parthipan <i>et al.</i> (2015)
11.	6-Acetyl-Beta.-D-Mannose	Antimicrobial agents	Mohammed <i>et al.</i> (2016)
12.	4-Ethyl-2-Hydroxycyclopent-2-en-1-one	Antibacterial activity	Ara (2012)
13.	Cyclopropanetetradecanoic Acid, 2-Octyl-, Methyl Ester	Antimicrobial	Srivastava <i>et al.</i> (2015)

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917 **Table 7:** FTIR analysis of p.ether, acetone and ethanol leaf extract of *Smilax zeylanica*

Serial number	Peak values cm ⁻¹	Functional group vibration	Expecting chemical compound
1.	3352	Strong O-H Stretching vibration	Alcohol group
2.	2962	Medium CH vibration	Amine group
3.	1707	Strong C=O Stretching vibration	Carbocyclic acids
4.	1366	Medium O-H bending vibration	Phenol
5.	1229	Strong C=O Stretching vibration	Ether
6.	1027	Strong C-O Stretching vibration	Alkyl aryl ether
7.	681	C=C bending vibration	Alkene

918 **Table 8:** Phytochemical compounds of *Smilax zeylanica* extracted with petroleum ether, acetone and ethanol.

S. No.	Compound Name	Pubchem ID
1.	Phytol	5280435
2.	3,7,11,15Tetramethyl-2-Hexadecen-1-ol	5366244
3.	Pentadecanoic Acid	13849
4.	1-Hexyl-2-Nitrocyclohexane	544017
5.	E-3-Pentadecen-2-ol	5363322
6.	Hexadecane, 1-Chloro-	20993
7.	Tritetracontane	522398
8.	Vitamin E	14985
9.	2-(2-Piperidin-1-yl-Ethyl)-Pyridine -	79548
10.	3-Acetoxydodecane	537256
11.	HexadecanoicAcid,2-Bromo-	82145
12.	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-Dihydroxy-1-Methoxy-7-(7-Methoxy-1,3-	101282029
13.	1-Monolinoleoylglycerol Trimethylsilyl Ether	5377604
14.	2-Pental, 2-Methyl-	5319754
15.	6-Methyl-7-oxo-1,7-Dihydro-Triazolo(4,3-B)Triazine	592014
16.	6-Acetyl-.Beta.-D-Mannose	439780
17.	4-Ethyl-2-Hydroxycyclopent-2-en-1-one	566236
18.	Cyclopropanetetradecanoic Acid, 2-Octyl-, Methyl Ester	552099
19.	D-Galactitol, 1,3,4,5-Tetra-O-Methyl-, Diacetate	538739
20.	Eicosanoic Acid, 2,3-bis[(Trimethylsilyl) oxy]Propyl Ester	537899
21.	11-Eicosenoic Acid, Trimethylsilyl Ester	5366418
22.	Ethanol,1-(Methylenecyclopropyl)-1-(Methylene-1 Trimethylsilylcy	552358
23.	4,4'-Biscyclohexanone, 2,2',6,6'-Tetramethyl-	536293

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Table 9: Docking Score and H Bond interactions of potential inhibitory compounds against *Aedes aegypti* and *Culex quinquefasciatus* D7 protein

S.No	Compound Id	Glide Score	No of H Bonds	Residues	Distance
1	439780	-8.75	8	GLU18, GLU18, ARG270, ARG15, THR14, ARG267, ARG267, ARG267	1.92, 1.87, 2.19, 2, 1.79, 2.1, 2.19, 2.2
2	101282029	-6.81	2	ARG198, ARG57	2.07, 1.92
3	538739	-5.31	3	LYS202, ARG198, ARG57	2.03, 1.86, 2.21
4	566236	-5.19	4	GLU18, GLU18, ARG15, ARG270	2.25, 2.08, 1.97, 2.05
5	552358	-4.23	1	LYS202	2.04

(Compound id- Pubchem database id, G-score – Docking score, No of H bonds – Number of H bonds involved in interaction, Residues – residues which is involved in interaction, Distance – distance of H bond)

Table 10: Docking Score and H Bond interactions of potential inhibitory compounds against *Anopheles stephensi* D7 protein

S.No	Compound Id	Glide Score	No of H Bonds	Residues	Distance
1	101282029	-7.24	2	ARG199, CYS55	2.13, 1.97
2	439780	-6.21	5	ARG199, ARG199, GLN11, GLY59, GLN61	2.06, 1.92, 2.14, 2.04, 2.31
3	14985	-5.07	1	MET201	2.09
4	552099	-4.57	2	ARG199, ARG199	1.96, 2.09
5	536293	-4.36	1	ARG199	1.99

Compound id- Pubchem database id, G-score – Docking score, No of H bonds – Number of H bonds involved in interaction, Residues – residues which is involved in interaction, Distance – distance of H bond)

933 **Table 11:** Docking Score and H Bond interactions of potential inhibitory compounds against *Aedes aegypti*
 934 Odorant protein

S.No	Compound Id	Glide Score	No of H Bonds	Residues	Distance
1	101282029	-10.89	1	HIE111	1.91
2	5366244	-10.23	2	HIE111, MET55	1.73, 2.1
3	5280435	-10.09	2	HIE111, MET55	1.73, 2.12
4	552099	-9.73	1	HIE111	1.96, 2.09
5	14985	-9.36	1	ARG199	1.99

935 (Compound id- Pubchem database id, G-score – Docking score, No of H bonds – Number of H bonds involved
 936 in interaction, Residues – residues which is involved in interaction, Distance – distance of H bond)

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939 **Table 12:** Docking Score and H Bond interactions of potential inhibitory compounds against *Culex*
 940 *quinquefasciatus* Odorant protein

S.No	Compound Id	Glide Score	No of H Bonds	Residues	Distance
1	101282029	-10.45	1	TRP114	2.04
2	14985	-10.19	1	MET201	2.16
3	536293	-8.36	1	ARG199	1.93
4	5366418	-8.12	1	HIE111, MET55	1.86, 1.73
5	5280435	-7.82	1	HIE111	2.09

941 (Compound id- Pubchem database id, G-score – Docking score, No of H bonds – Number of H bonds involved
 942 in interaction, Residues – residues which is involved in interaction, Distance – distance of H bond)

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949 **Table 13.**Adulticidal effect of petroleum ether leaf extract of *Smilax zeylanica* on the malarial, dengue and
950 filarial vectors

Targeted mosquito species	Mortality (%) (mean±SD)					LC ₅₀ ppm (LFL-UFL)	LC ₉₀ ppm (LFL-UFL)	χ^2
	Concentration(ppm)							
	100	200	300	400	500			
<i>An. stephensi</i>	27.0±2.23 ^a	39.2±1.92 ^b	51.6±2.07 ^c	66.4±1.81 ^d	74.7±2.22 ^e	284.92 (247.63-320.41)	678.15 (594.86-814.10)	0.220*
<i>Ae. aegypti</i>	23.2±2.38 ^a	36.4±2.07 ^b	48.6±1.92 ^c	62.8±2.28 ^d	72.0±2.54 ^e	311.97 (276.79-348.48)	700.05 (614.22-839.72)	0.245*
<i>Cx. quinquefasciatus</i>	21.6±2.07 ^a	33.0±1.41 ^b	45.4±2.30 ^c	59.6±2.60 ^d	70.8±3.03 ^e	332.76 (298.52-370.65)	715.34 (627.96-857.01)	0.034*

951 Mortality rates are means ± SD of triplicate replicates. No mortality was observed in the control. Within each
952 column means followed by the same letter(s) are not significantly different (P<0.05). LC₅₀ lethal concentration
953 that kills 50 % of the exposed organisms, LC₉₀ lethal concentration that kills 90 % of the exposed organisms,
954 LCL lower confidence limit, UCL upper confidence limit. Chi-square value followed by an asterisk is
955 significant (P<0.05)

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959 **Table 14:** Adulticidal effect of acetone leaf extract of *Smilax zeylanica* on the malarial, dengue and filarial
960 vector.

Targeted mosquito species	Mortality (%) (mean±SD)					LC ₅₀ ppm (LFL-UFL)	LC ₉₀ ppm (LFL-UFL)	χ^2
	Concentration(ppm)							
	100	200	300	400	500			
<i>An. stephensi</i>	31.4±2.07 ^a	45.6±2.40 ^b	56.8±2.38 ^c	71.0±1.22 ^d	79.2±1.92 ^e	241.97 (199.70-277.31)	634.40 (557.76-759.05)	0.213*
<i>Ae. aegypti</i>	27.6±2.07 ^a	42.2±1.92 ^b	53.1±1.67 ^c	67.5±1.93 ^d	76.4±1.81 ^e	271.61 (233.31-306.57)	662.33 (581.90-793.11)	0.273*
<i>Cx. quinquefasciatus</i>	24.2±1.92 ^a	38.1±2.40 ^b	49.4±1.67 ^c	63.2±2.16 ^d	72.3±2.86 ^e	304.99 (268.61-341.93)	703.03 (614.86-848.18)	0.293*

961 Mortality rates are means ± SD of triplicate. No mortality was observed in the control. Within each column
962 means followed by the same letter(s) are not significantly different (P<0.05). LC₅₀ lethal concentration that kills
963 50 % of the exposed organisms, LC₉₀ lethal concentration that kills 90 % of the exposed organisms, LCL lower
964 confidence limit, UCL upper confidence limit. Chi-square value followed by an asterisk is significant
965 (heterogeneity factor used in calculation of confidence limits) (P<0.05)

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970 **Table 15:** Adulticidal effect of ethanol leaf extract of *Smilax zeylanica* on the malarial, dengue and filarial
971 vector

Targeted mosquito species	Mortality (%) (mean±SD)					LC ₅₀ ppm (LFL-UFL)	LC ₉₀ ppm (LFL-UFL)	χ^2
	Concentration(ppm)							
	100	200	300	400	500			
<i>An. stephensi</i>	33.1±2.07 ^a	49.4±2.40 ^b	60.2±1.92 ^c	74.7±2.38 ^d	84.0±1.58 ^e	217.12 (174.75-251.02)	578.86 (514.60-679.35)	0.257*
<i>Ae. aegypti</i>	30.0±2.00 ^a	45.8±1.78 ^b	57.4±2.07 ^c	71.3±2.22 ^d	81.5±1.51 ^e	241.89 (202.80-275.02)	607.15 (538.79-714.60)	0.176*
<i>Cx. quinquefasciatus</i>	28.5±1.61 ^a	41.2±2.16 ^b	54.0±2.12 ^c	67.6±2.07 ^d	78.8±1.92 ^e	266.90 (229.70-300.55)	642.43 (567.51-761.88)	0.013*

972 Mortality rates are means ± SD of triplicate. No mortality was observed in the control. Within each column
973 means followed by the same letter(s) are not significantly different (P<0.05). LC₅₀ lethal concentration that kills
974 50 % of the exposed organisms, LC₉₀ lethal concentration that kills 90 % of the exposed organisms, LCL lower
975 confidence limit, UCL upper confidence limit. Chi-square value followed by an asterisk is significant
976 (heterogeneity factor used in calculation of confidence limits) (P<0.05)

998 **Table 16:** Repellent activity of of p.ether, acetone and ethanol leaf extracts of *Smilax zeylanica* against *An.*
999 *stephensi*

Plant solvent extracts	Concentration (mg/cm ²)	% of Repellency ± SD					
		Time post application of repellent (min)					
		60	90	120	150	180	210
Petroleum ether	0.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	87.6±1.34
	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.4±2.07
	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Acetone	0.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.2±0.83
	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.6±2.07
	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Ethanol	0.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.7±0.8
	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.2±1.48
	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

1000 Values were means±SD of five replicates.

Table 17: Repellent activity of p.ether, acetone and ethanol leaf extracts of *Smilax zeylanica* against *Ae. aegypti*

Plant solvent extracts	Concentration (mg/cm ²)	% of Repellency $\pm$ SD					
		Time post application of repellent (min)					
		60	90	120	150	180	210
Petroleum ether	0.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	82.3 $\pm$ 1.34
	1.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	92.1 $\pm$ 2.07
	1.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
Acetone	0.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	87.8 $\pm$ 0.83
	1.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	94.4 $\pm$ 2.07
	1.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
Ethanol	0.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	91.2 $\pm$ 2.07
	1.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	95.3 $\pm$ 1.48
	1.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0

Values were means $\pm$ SD of five replicates.

Table 18: Repellent activity of p.ether, acetone and ethanol leaf extracts of *Smilax zeylanica* against, *Cx. quinquefasciatus*

Plant solvent extracts	Concentration (mg/cm ²)	% of Repellency $\pm$ SD					
		Time post application of repellent (min)					
		60	90	120	150	180	210
Petroleum ether	0.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	90.5 $\pm$ 1.34	81.2 $\pm$ 1.34
	1.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	96.1 $\pm$ 2.07	89.4 $\pm$ 2.07
	1.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	93.2 $\pm$ 1.48
Acetone	0.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	93.1 $\pm$ 0.83	84.6 $\pm$ 0.83
	1.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	98.8 $\pm$ 2.07	91.3 $\pm$ 2.07
	1.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	95.3 $\pm$ 1.34
Ethanol	0.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	89.4 $\pm$ 2.07
	1.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	97.2 $\pm$ 1.48
	1.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0

Values were means $\pm$ SD of five replicates.

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GRAPHICAL ABSTRACT

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Topic: Molecular interaction of novel phytocompounds from *Smilax zeylanica* (L) against the targeted protein of mosquito vectors and their potential activity

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