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Molecular docking studies of phytoconstituents extracted from the *Calotropis gigantea* and *Strobilanthes kunthiana* plants against the target protein carboxylic ester hydrolase from *Culex quinquefasciatus* and drug design strategies

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Abstract
Plant-based phytoconstituents have been in use against larvicidal, pupicidal, and repellent actions, which have gained increasing popularity in recent days and are safe when compared to synthetic insecticides. In this study, seven ligands were found from *C. gigantea* and *S. kunthiana* leaf ethanol extract using GCMS. The target protein, carboxylic ester hydrolase, from *Cx quinquefasciatus*, was taken from the UniProt database. It was predicted the 3D structure of the target protein and the sequence identity was 69.49%. The best model was chosen and evaluated using the Ramachandran plot. 88.2% of residues are located in the most favoured regions, which confirm that the predicted model is good. According to molecular docking studies, all seven compounds interacted with the target protein. Among the 7 compounds, Neophytadiene (-6.2 Kcal/mol) showed very good interaction with the carboxylic ester hydrolase (target protein). Hexadecanoic acid showed the lowest binding affinity (-5.3 Kcal/mol) with the amino acid residues of the target protein. The ADMET and CYP properties were tested for the phyto-compound Neophytadiene. The good binding affinity of phytocompound neophytadiene obeys the Lipinski rule of five, and the compound did not cross the blood—brain barrier (BBB) and has low intestinal absorption (HIA). The fractional CSP3 value is within the range of rotatable bonds of the compound. The CYP property of the compound does not inhibit most of the CYP450 enzymes and does not cause any adverse reactions, but it inhibits only the CYP2C9 enzyme. The neophytadiene value of log Kp (Skin Permeant) is -1.17, showing less skin permeant, and a bioavailability Score (ABS) of 0.55 shows that it passes the rule of five.

Keywords: molecular docking, conformation, ligand, receptor, optimization, ADMET and CYP

Introduction
Plants provide biologically active compounds; some compounds are useful for human metabolic activity; some other compounds have toxic properties that are active against pathogenic organisms; and still other compounds are useful for the preparation of drugs for the treatment of some diseases. Most of the plants have medicinal value, and they contain a wide range of bioactive compounds that have anticancer, anti-inflammatory, antimicrobial, and antioxidant potential (Ralte *et al.*, 2022) [5]. Mosquitoes can transmit more life-threatening diseases like malaria, filariasis, yellow fever, dengue fever, chikungunya fever, Zika virus fever, West Nile fever, Japanese encephalitis, etc., in almost all tropical and subtropical countries and many other parts of the world (WHO, 2020) [7]. Mosquito control is essential to prevent the proliferation of these diseases. Therefore, the application of synthetic insecticides has not been very successful due to human, technical, operational, ecological, and economic factors (Ghosh *et al.*, 2012) [6]. This concept has resulted in an urge to look for environmentally friendly, cost-effective, biodegradable, and target-specific insecticides against mosquito species. Bearing this in mind, the application of eco-friendly alternatives such as biological control of mosquitoes has become the central focus of this study in lieu of synthetic insecticides.

In this study, two plant extracts such as *Calotropis gigantea* and *Strobilanthes kunthiana* were used as insecticides for killing larvae and adult mosquitoes for protection against mosquito bites. Kavitha *et al.* (2015) [9] highlighted the presence of flavanoids, phenolic compounds, tannins, steroids, glycosides, and triterpenoids in *Strobilanthes ciliatus*. The plant species *C. gigantea* and *S. kunthiana* contain a vast pool of secondary metabolites. To date, most of the studies have analysed *C. gigantea* metabolites, but no one has analysed or studied and proven *S. kunthiana* plant, which is a substantial source of bioactive compounds. These compounds were analysed for the associations between biologically relevant molecules that play a central role in signal transduction. Knowledge of the preferred orientation is important to predict the strength of association or binding affinity between the molecules. The integration of computational and experimental strategies has been of great value in the identification and development of novel promising compounds (Ferreira *et al.*, 2015) [8]. The investigation of modern bioactive compounds has molecular docking methods to explore the ligand conformations adopted within the binding sites of macromolecular targets. This approach also estimates the ligand-receptor binding free energy by evaluating critical phenomena involved in the intermolecular recognition process. Today, as a variety of docking algorithms are available, an understanding of the advantages and limitations of each method is of fundamental importance in the development of effective strategies and the generation of relevant results. A molecular docking technique is used to predict the tentative binding parameters of ligand-receptor complexes.

According to Lengauer and Rarey (1996) [4], molecular docking is a method that predicts the preferred orientation of one molecule towards a second when bound to each other to form a stable complex. Molecular docking is the process in which two molecules fit together in 3D space and is a key tool in structural biology and computer-aided drug design (Dutkiewicz, 2020) [21]. Docking studies were analysed to determine how two or more molecular structures fit together. Therefore, molecular docking is useful for predicting both the molecular strength of binding and the type of signal produced between the plants *C. gigantea* and *S. kunthiana* bioactive compounds and mosquito target protein molecules. The main aim of the present study is to prevalent ligands from *C. gigantea* and *S. kunthiana* plants and the target protein were extracted from leaves. The 3D structure of the target protein was predicted and the best model was evaluated using the Ramachandran plot. Further, the molecular docking studies revealed that target protein and ligand interactions were evaluated. The ADMET and CYP properties were tested for the neophytodiene phyto-compounds.

Materials and Methods

Ligand selection

The 3D structures of the phytocompounds, which include Neophytadiene, Phytol, 9,12,15-Octadecatrienoic acid (Z, Z, Z), 9,12-Octadecadienoic acid, N-Hexadecanoic acid, Lidocaine, and Hexadecanoic acid ethyl ester, were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Target protein selection and preparation

The sequence of target protein carboxylic ester hydrolase from *Culex quinquefasciatus* was taken from the UniProt database (https://www.uniprot.org). It was modelled using SWISS–MODEL (Waterhouse *et al.*, 2018) to predict the 3D structure of the target protein, the best model was taken and evaluated using the Ramachandran plot (https://saves.mbi.ucla.edu/). The modelled 3D structure of the target protein, carboxylic ester hydrolase, was prepared using Discovery Studio 2021 for in silico docking studies.

Docking studies

Docking studies for the target protein carboxylic ester hydrolase and phyto-compounds (ligands) were done using PyRx 0.8 software (Trott and Olson, 2010) [2]. The target protein was further prepared for docking studies using this software. All of the ligands were uploaded using PyRx 0.8's Open Babel option. The grid was created and the docking studies were carried out in PyRx 0.8 using the Vina wizard option. The values of binding affinity were saved in the XL file. The results were analysed using Discovery Studio 2021, and the 2D and 3D docked images were taken. In the results, the lowest binding affinity indicates a good result.

ADMET and CYP properties

CYP and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties were tested for the phytocompound neophytadiene using Swiss ADME (Daina *et al.*, 2017) [3]. The compound was evaluated for XLogP3, TPSA (Topological Polar Surface Area), Log S, Fraction Csp3, Rotatable Bonds, CYP properties, skin permeation, and bioavailability score.

Results

Ligand and target protein selection

The identified ligands (phytocompounds) 3D structures were retrieved from the PubChem database. The sequence of target protein carboxylic ester hydrolase from *C. quinquefasciatus* was taken from the UniProt database and its UniProt ID is B0W0J3. It was modelled using SWISS–MODEL and Acetylcholinesterase (PDB ID: 6XYY) was used as a template to predict the 3D structure of the target protein and the sequence identity is 69.49%. The 3D structure of the target protein was evaluated using a Ramachandran plot. In the results, 88.2% of residues are located in the most favoured regions, which confirm that the predicted model is good. The other regions of Ramachandran plot statistics implied that the modelled 3D structure has 10.5% of its residues in additional allowed regions, 1.1% of its residues in the generously allowed regions and 0.2% of its residues in disallowed regions of the Ramachandran plot. This also validates that the modelled 3D structure is a good quality model. The 3D structure of the modelled target protein is shown in Figure 1, and the Ramachandran plot for the target protein is shown in Figure 2.

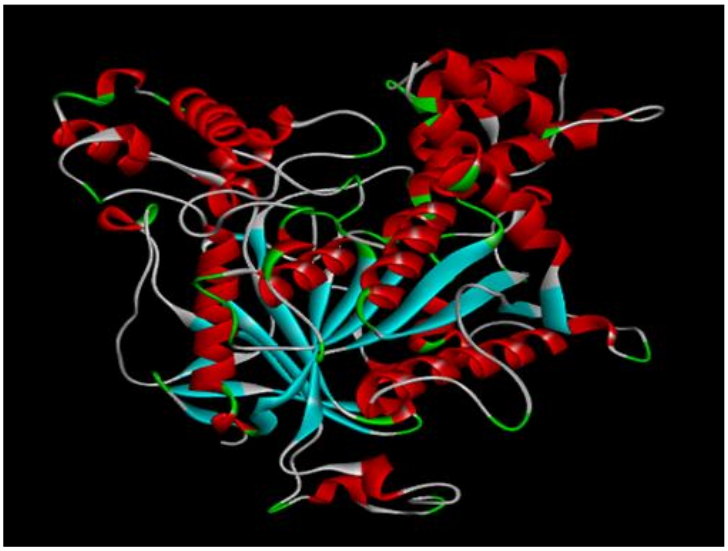

Fig 1: The 3D structure of modelled target protein Carboxylic ester hydrolase

Docking studies

Docking studies for the target protein carboxylic ester hydrolase and phytocompounds (ligands) were done using PyRx 0.8 software. In the results, all the 7 compounds interacted with the target protein. The results are shown in table 1, and the 2D and 3D interactions of phytocompounds with the target protein are shown in figures 3–16.

Table 1: Interaction of Phytocompounds with the target protein Carboxylic ester hydrolase

S. No.	PubChem (CID)	Compound Name	Binding Affinity (Kcal/mol)	No. of Bonds	Interacting Residues	Bond Length (Å)
1	10446	Neophytadiene	-6.2	11	TYR 392 TYR 388 PHE 389 TYR 342 TRP 339 TRP 339 TRP 339 VAL 336 VAL 336 TYR 95 TYR 95	4.87 4.46 4.84 4.09 4.65 3.71 4.37 4.03 4.89 4.63 3.74
2	5280435	Phytol	-5.9	14	TYR 342 ASP 393 TYR 392 TYR 97 TYR 95 TYR 95 VAL 336 TRP 339 TRP 339 TRP 339 TRP 339 TRP 339 TRP 339 TRP 339	3.81 3.37 2.35 2.93 4.94 3.63 4.27 4.83 4.38 4.61 3.56 4.89 4.89 4.55
3	5280934	9,12,15-Octadecatrienoic acid, (Z, Z, Z)	-5.8	10	TYR 392 TYR 388 PHE 389 TYR 342 TYR 342 TYR 342 TRP 339 TRP 339 TYR 95 TYR 97	5.17 5.07 4.99 4.87 5.17 5.00 4.26 3.57 3.76 4.90
4	5280450	9,12-Octadecadienoic acid	-5.6	10	VAL 336 TYR 95 TYR 95 TRP 339 TRP 339 TRP 339 TRP 339 TRP 339 ASP 393 TYR 342	4.24 4.50 5.25 4.20 5.37 4.69 4.61 4.90 2.14 4.59
5	985	N-Hexadecanoic acid	-5.5	9	TYR 95 VAL 336 VAL 336	5.02 4.26 4.44

					TRP 339	4.78
					TRP 339	4.22
					TRP 339	3.79
					TRP 339	4.87
					TYR 342	3.77
					ASP 393	2.83
6	3676	Lidocaine	-5.5	6	TYR 342	5.05
					TRP 339	4.10
					TRP 339	3.93
					TRP 339	4.75
					GLU 93	3.76
					VAL 336	3.80
7	12366	Hexadecanoic acid, ethyl ester	-5.3	10	TYR 388	5.42
					PHE 389	5.21
					PHE 348	5.48
					TRP 339	3.67
					TRP 339	5.04
					TRP 339	4.28
					TRP 339	4.11
					TYR 95	4.80
					TYR 95	3.55
					TYR 97	5.09

From the results (Table 1), among the seven phytocompounds, neophytadiene showed very good binding affinity (-6.2 Kcal/mol) with the amino acid residues TYR 392, TYR 388, PHE 389, TYR 342, TRP 339, VAL 336, and TYR 95 of the target protein. The compound Phytol showed a good binding affinity of -5.9 Kcal/mol with the amino acid residues like TYR 342, ASP 393, TYR 392, TYR 97, TYR 95, VAL 336, and TRP 339. The phyto-compound 9,12,15-Octadecatrienoic acid (Z, Z, Z) showed 10 bonds with the amino acid residues TYR 392, TYR 388, PHE 389, TYR 342, TRP 339, TYR 95, and TYR 97, and its binding affinity is -5.8 Kcal/mol. Further, among the 7 compounds, hexadecanoic acid, ethyl ester showed the lowest binding affinity (-5.3 Kcal/mol) with the amino acid residues TYR 388, PHE 389, PHE 348, TRP 339, TYR 95, and TYR 97 of the target protein.

Hence, the present study concludes that the phytocompound neophytadiene showed very good interaction with the target protein carboxylic ester hydrolase and may have the potential to control the population of *Cx. quinquefasciatus*.

Table 2: ADMET Properties of Phyto-compound

S. No	PubChem (CID)	Compound Name	Lipinski	BBB	HIA	PGP (-)	XLOGP3	TPSA (Å)	Log S (ESOL)	Fraction Csp3	Rotatable Bonds
1	10446	Neophytadiene	Yes	No	Low	No	9.62	0.00	-6.77	0.80	13

Note: Lipinski: 'Yes' means, it obeys the Lipinski Rule of Five & good and 'No' means, has Violation; BBB (Blood - Brain Barrier): 'Yes' means good, HIA (Human Intestinal Absorption): 'High' means good; PGP (-): 'Yes' means good & Molecules predicted not to be effluated from the CNS by P-glycoprotein and 'No' means, Molecules predicted to be effluated from the CNS by P-glycoprotein; Lipophilicity (XLOGP3): The value is between '−0.7 and +5.0' means good; Polarity (TPSA): The value is between 20 and 130 Å means good; Water Solubility (Log S): The value is not higher than 6 means good; Saturation (Fraction Csp3): Fraction of carbons in the sp3 hybridization is not less than 0.25 means good; and Flexibility (Rotatable bonds): No more than 9 rotatable bonds means good.

Table 3: Cytochrome P450 properties of Phyto-compound

S. No	PubChem (CID)	Compound Name	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log K_p (Skin permeation) (cm/s)	A Bioavailability Score (ABS)
1	10446	Neophytadiene	No	No	Yes	No	No	-1.17	0.55

Note: 'No' means, Good & the compound does not inhibit the CYP450 enzymes and does not give any adverse reactions; 'Yes' means, the compound inhibits the CYP450 enzymes and gives unanticipated adverse reactions; The more negative the log K_p value means, the molecule is the less skin permeant; ABS 0.55 means, it passes the rule of five & -1.17 means, it fails the rule of five.

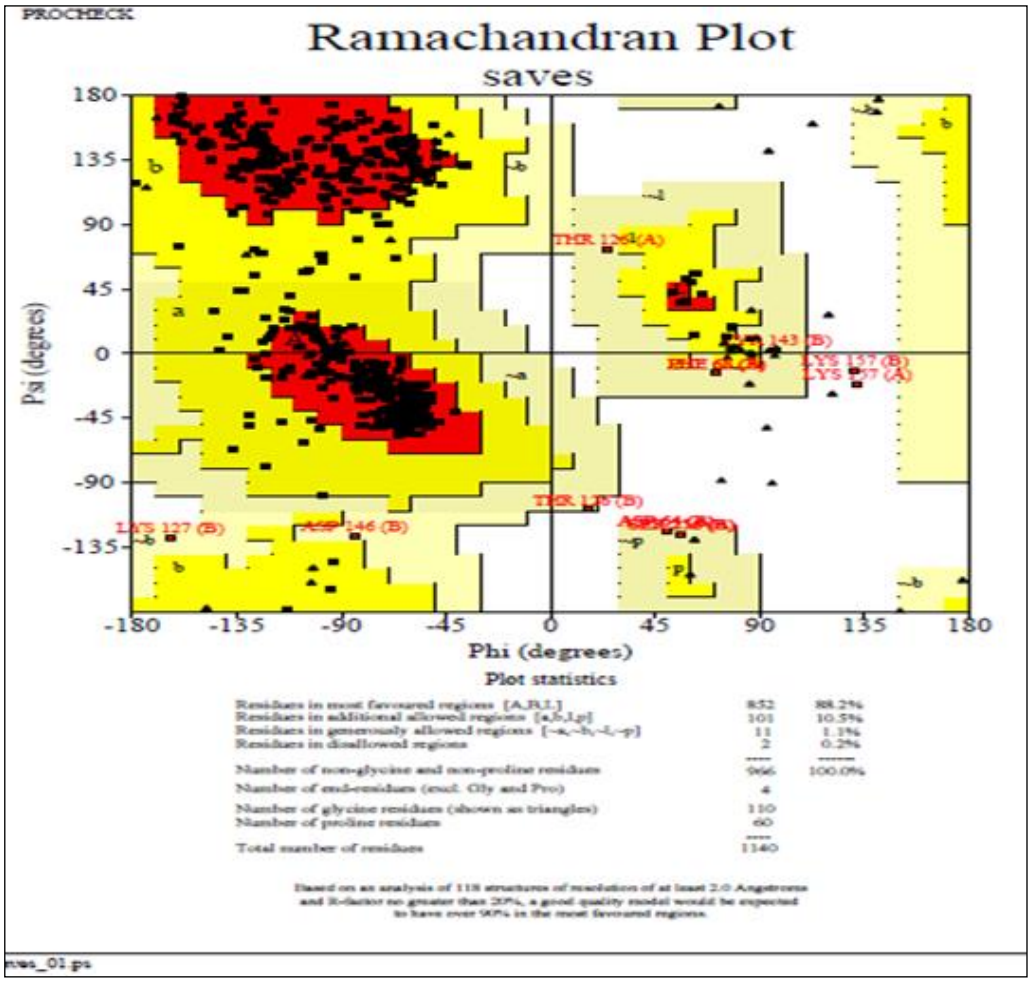

Fig 2: Ramachandran plot for the target protein Carboxylic ester hydrolase

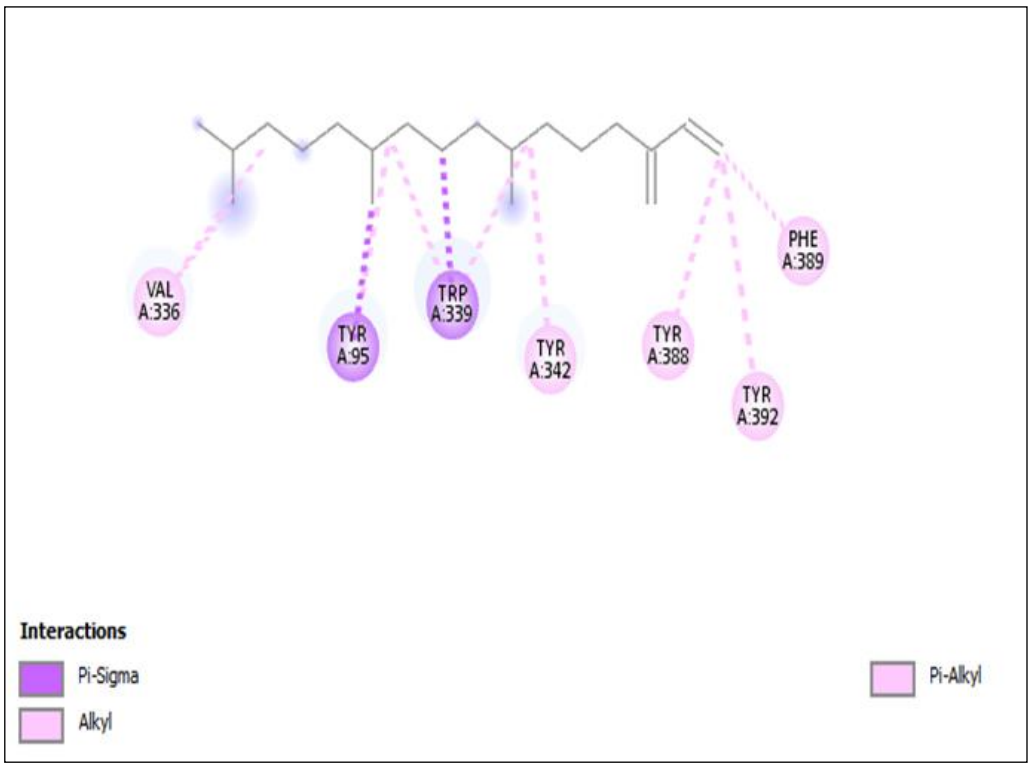

Fig 3: The 2D interaction of phytocompound Neophytadiene with the target protein

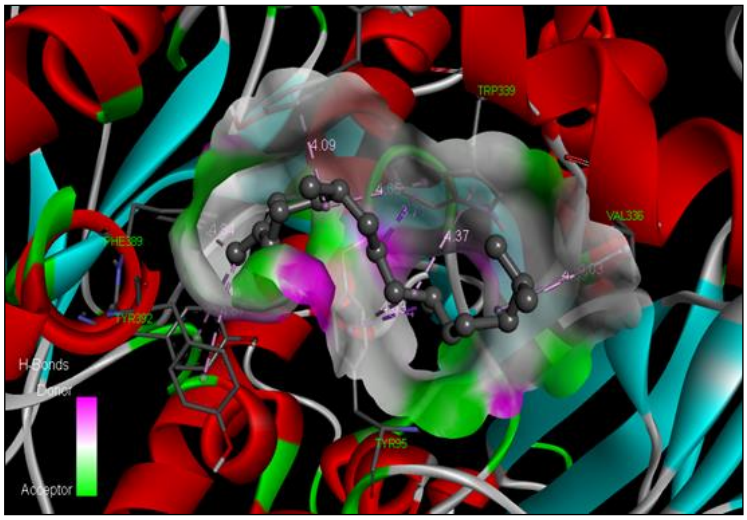

Fig 4: The 3D interaction of phytocompound Neophytadiene with the target protein

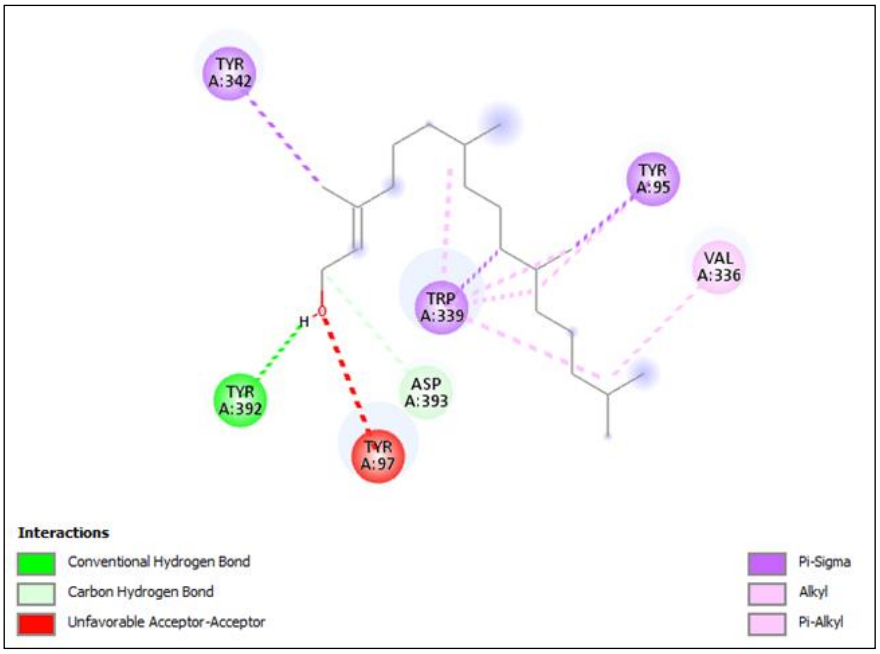

Fig 5: The 2D interaction of phytocompound Phytol with the target protein

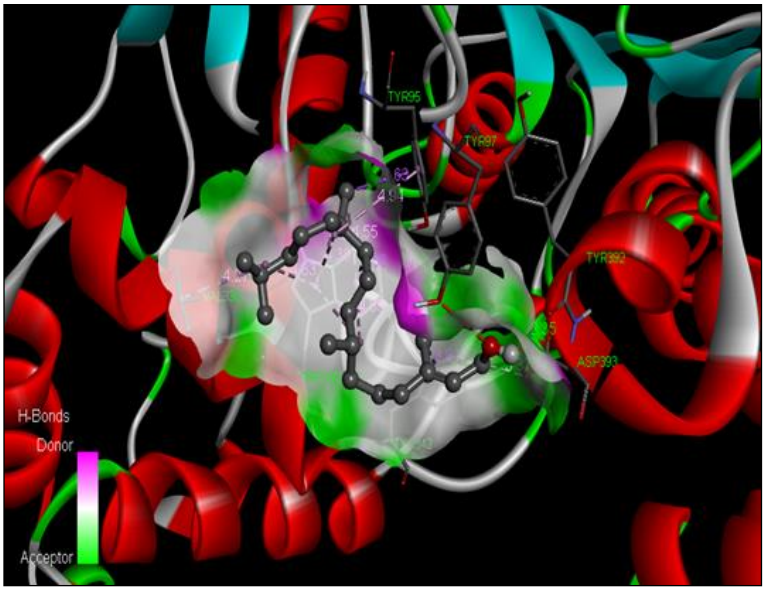

Fig 6: The 3D interaction of phytocompound Phytol with the target protein

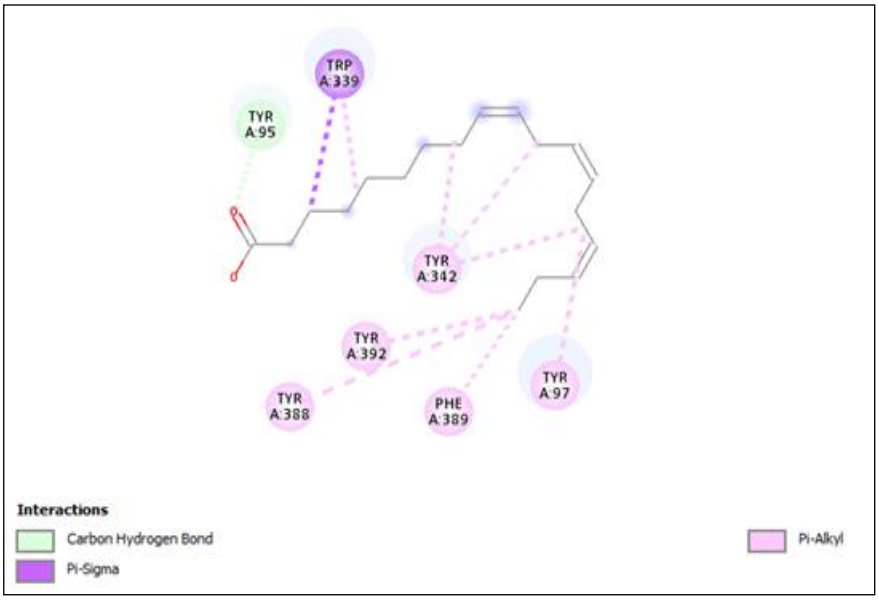

Fig 7: The 2D interaction of phytocompound 9,12,15-Octadecatrienoic acid, (Z, Z, Z) with the target protein

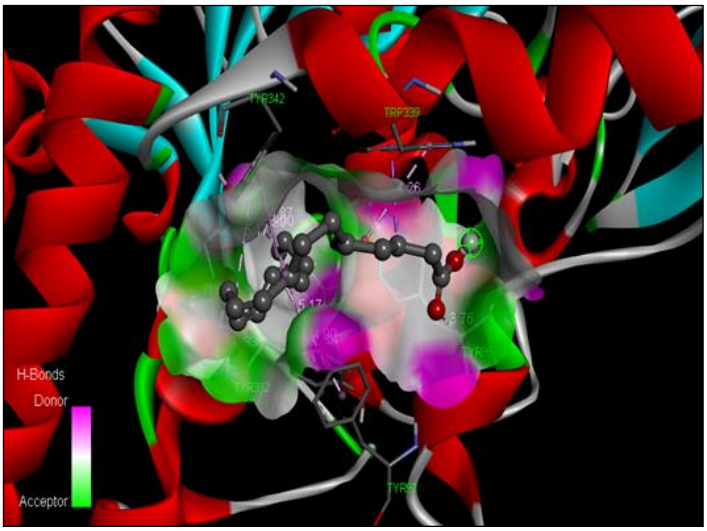

Fig 8: The 3D interaction of phytocompound 9,12,15-Octadecatrienoic acid, (Z, Z, Z) with the target protein

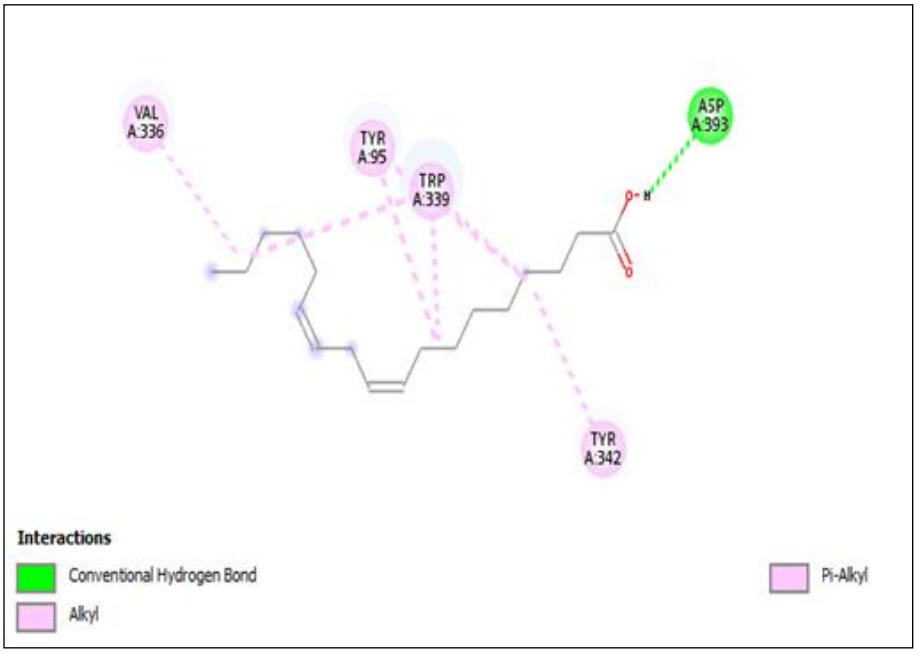

Fig 9: The 2D interaction of phytocompound 9,12-Octadecadienoic acid with the target protein

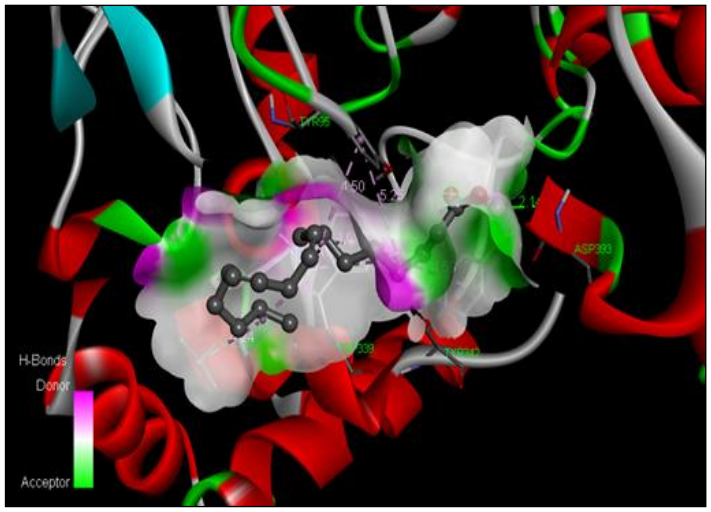

Fig 10: The 3D interaction of phytocompound 9,12-Octadecadienoic acid with the target protein

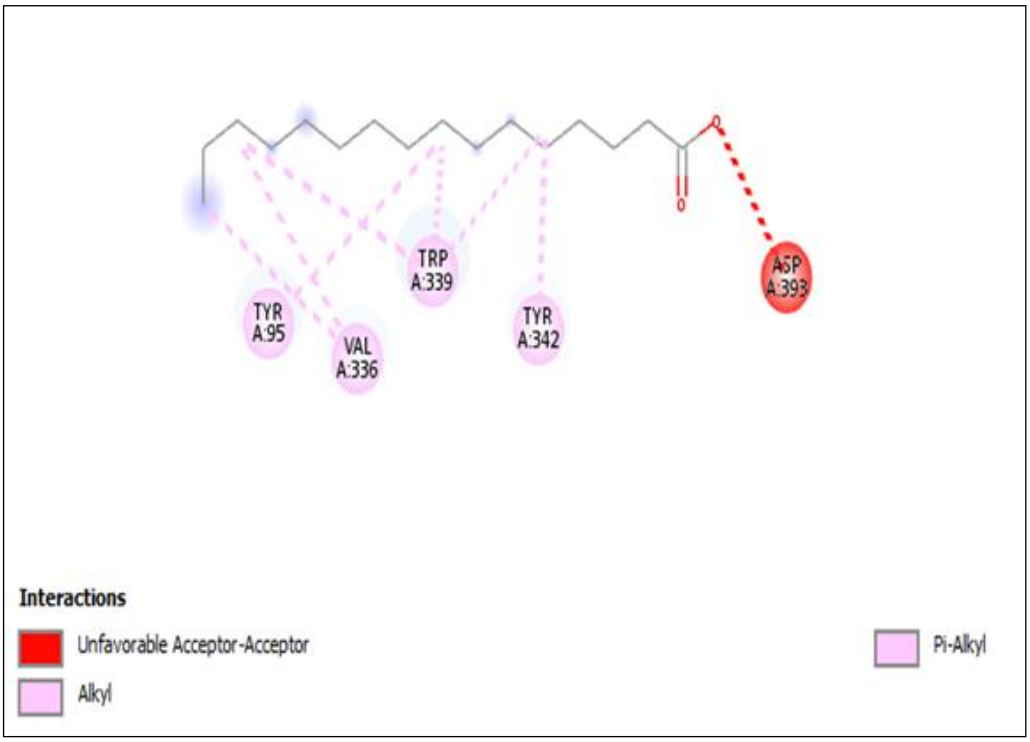

Fig 11: The 2D interaction of phytocompound N-Hexadecanoic acid with the target protein

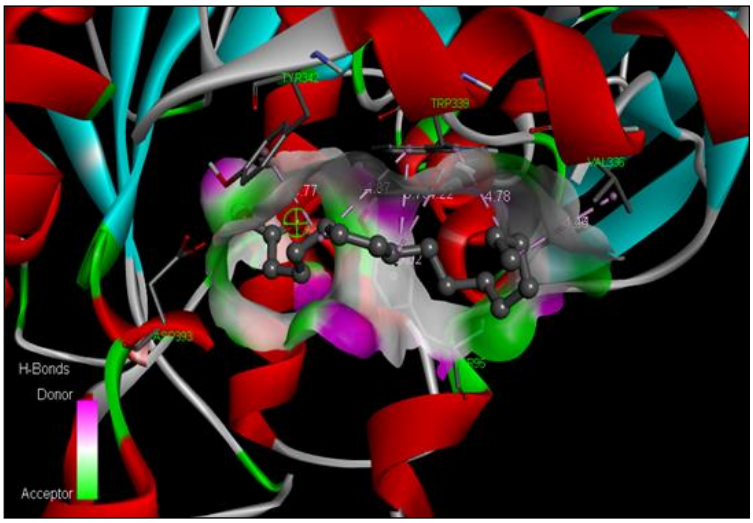

Fig 12: The 3D interaction of phyto-compound N-Hexadecanoic acid with the target protein

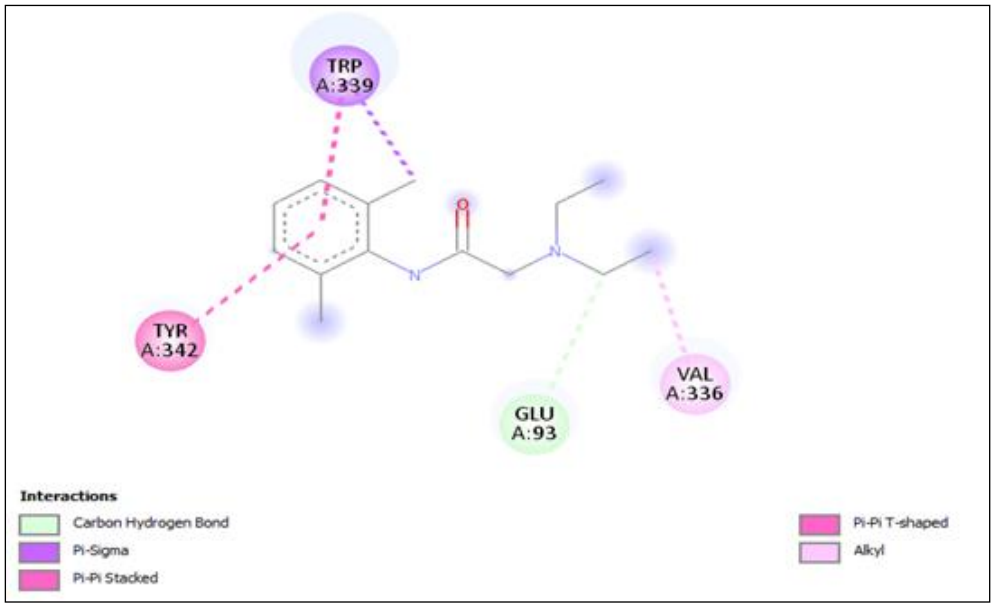

Fig 13: The 2D interaction of phyto-compound Lidocaine with the target protein

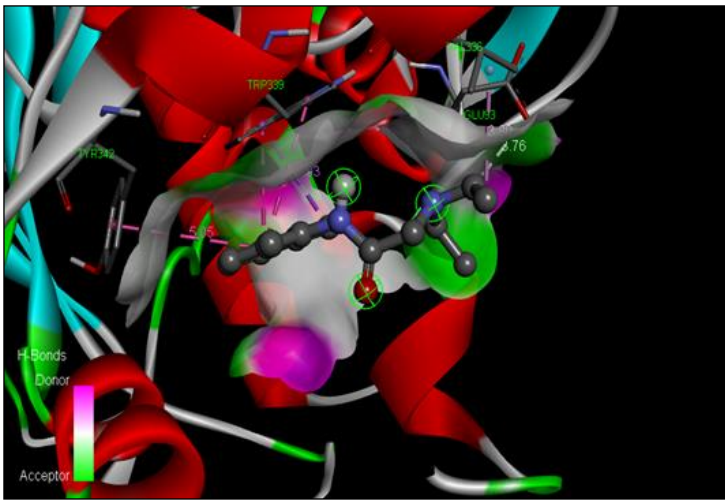

Fig 14: The 3D interaction of phytocompound Lidocaine with the target protein

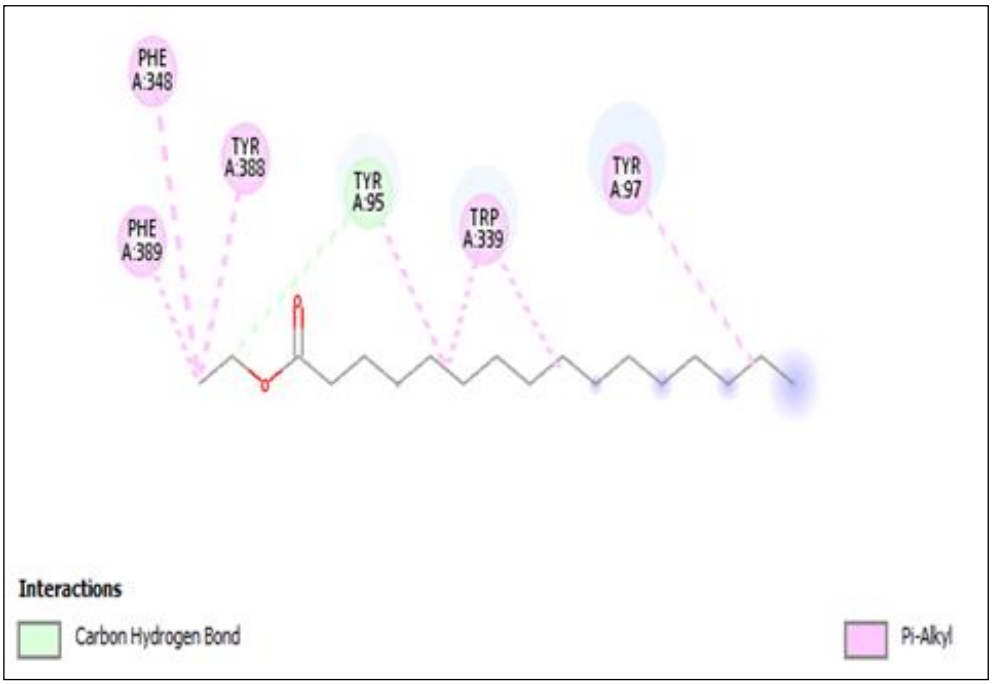

Fig 15: The 2D interaction of phyto-compound Hexadecanoic acid, ethyl ester with the target protein

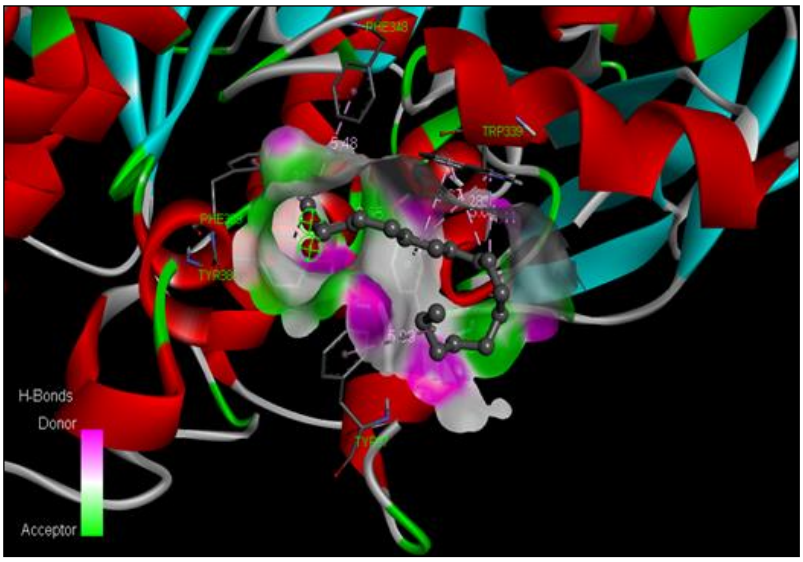

Fig 16: The 3D interaction of phyto-compound Hexadecanoicacid, ethyl ester with the target protein

ADMET and CYP properties

The ADMET and CYP properties of phyto-compounds were tested for the best binding affinity with target protein using Swiss ADME, and the results are tabulated in tables 2 and 3. From the results of ADMET properties (table 2), the best compound, neophytadiene, obeys the Lipinski rule of five, and the compound did not cross the Blood–Brain Barrier (BBB) and has low intestinal absorption (HIA). The compound is predicted to be effluent from the CNS by P-glycoprotein. The XLogP3 value of the compound is above the range. The TPSA (Topological Polar Surface Area) value is not in the range and the Log S value of the compound is within the limit. In the compound, the fraction Csp3 value is within the range and the rotatable bonds of the compound are not in the limit.

In the results of CYP properties (table 3), the compound does not inhibit most of the CYP450 enzymes and does not cause any adverse reactions, but it inhibits only the CYP2C9 enzyme. The values of log Kp (Skin Per Mean) and bioavailability score (ABS) are good for the best compound, neophytadiene.

Discussion

Plant products are gaining importance in the discovery of phyto-compounds against mosquito control programmes. *C. gigantea* plants gain the attention of researchers because of their essential phyto-compounds possessing larvicidal, pupucidal, and mosquito repellent properties (Kumar *et al.*, 2012; Amjad Beg *et al.*, 2020) [26, 27]. Similarly, in this study, *S. kunthiana* volatile compounds were also extracted and used for mosquito repellent properties. Although computational algorithm methods are well documented in medicinal synthetic chemistry, their application in the field of natural phytocompounds is limited and unexplored. The molecular docking study contributes to the prediction of the ligand-receptor complex structure by computational approaches (Saravanan *et al.*, 2022) [22]. The molecular docking studies reveal the presence of novel compounds by comparing their highest binding affinity to receptor protein carboxylic ester hydrolase from *Cx. quinquefasciatus*.

The intermolecular forces affect the docking like bond width, bond angle, dihedral angle and intermolecular forces, which include electrostatic, dipolar, hydrogen bonding and hydrophobicity (Duran-Iturbide *et al.*, 2020) [18]. Swiss Dock is an online protein-ligand docking service where users can perform rigid-flexible docking by uploading protein and ligand files and can define the region of interest by entering centre coordinates and size (dimensions) of the grid box (Grosdidier *et al.*, 2011; Szalay and Rohonczy, 2011) [10, 11]. Depending upon the docking calculation results, the best predicted binding affinities do not vary significantly, being in the range of -6.2 to -5.3 kcal/mol, which showed hydrogen bond interactions and also electrostatic interactions of the target protein (TYR 392, TYR 388, PHE 389, TYR 342, TRP 339, VAL 336, and TYR 95). The results are quantified in terms of the lowest estimated free energy of binding conformation and the experimental binding mode. The presence of a hydrogen bond indicates that the ligand had a high binding affinity for the protein, whereas a high negative score indicates that the ligand had a low binding affinity for the target protein (Issa *et al.*, 2017). Based on the docking score that was gauged, making use of docking into the energetic site of the phytocompounds strongly recommends strong communication between neophytadiene and the carboxylic ester hydrolase, from which it is concluded that it, is lethal to *Cx. quinquefasciatus*.

The current finding for phytocompound neophytadiene is in good agreement with the results of Mamudha and Sunilson, (2021), who reported that phytocompound neophytadiene is lethal to Anopheles larvae development. It could be used as a larvicide as it is found to be a safe and efficient alternative for mosquito larval control. A Ramachandran plot is the phi/psi dihedral angles between N-Cα and Cα-C planar peptide bonds in a protein's backbone. According to Ramachandran plots for over 118 structures at 2.0 Å resolutions, a good quality model can be obtained when more than 90% of residues fall into the most preferred region (Al-Khayyat and Al-Dabbagh, 2016) [24].

Based on the Swiss ADME results, we conclude that the evaluation of ADMET in the exploration phase considerably lowers the faltering connected to pharmacokinetics in the scientific stage. These results were acknowledged by the results of Hay *et al.* (2014) [12]. Therefore, the computer-based prognosis of ADMET from the molecular structure is a legitimate choice for experiential methods (Dahlin *et al.*, 2015) [19]. In this research, Neophytadiene, Phytol, 9,12,15-Octadecatrienoic acid (Z, Z, Z), hexadecanoic acid, ethyl

ester, has the greatest lethal effect due to its low molecular weight, which enhances the permeation of mosquitoes control as well as reduced hydrogen-bonding ability, leading to more significant permeability in the structure (Haritha *et al.,* 2021) [20]. Neophytadiene obeys the Lipinski rule of five, and the compound did not cross the Blood—Brain Barrier (BBB) and has low intestinal absorption (HIA). The compound is predicted to be effluent from the CNS by P-glycoprotein. The XLogP3 value of the compound is above the range. The TPSA (Topological Polar Surface Area) value is not in the range and the Log S value of the compound is within the limit. In the compound, the fraction Csp3 value is within the range and the rotatable bonds of the compound are not in the limit. These results confirm that neophytadiene is an ideal choice for the control of *Cx. quinquefasciatus*. Our results were confirmed by the results of Nagamalla Sravika *et al.,* (2021) [25], who studied the individual ADME behaviours of the phytoconstituents (including neophytadiene) in *Bauhinia acuminate.*

The cytochrome P450 mixed oxidase enzyme's major isoenzymes play an integrative role in drug metabolism and its elimination in biological systems. About 80% of the molecules identified in the present study are substrates of five isoforms: CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. These results coincided with those of Stegemann *et al.* (2007). The bioavailability score of compound neophytadiene (ABS) was 0.55, which was good. These compounds do not inhibit the CYP450 enzymes and do not cause any adverse reactions. The inhibition of the CYP isomers by these compounds can cause poor bioavailability due to metabolic derangements and toxic side effects due to their accumulation (Srimai *et al.,* 2013). A few compounds in the present study inhibit the CYP450 enzymes and cause unanticipated adverse reactions. Inhibition of these isoenzymes is a major concern in pharmacokinetics-related phytocompound interactions and accumulation, leading to toxic ADME of the phytoconstituents and their metabolites (Wang *et al.,* 2015) [15], and the skin permeability coefficient (Log Kp) of Lipinski *et al,* (1996) [16]. The less skin permeant the molecule, the more negative the log Kp (with Kp in cm/s). Among the phytocompounds, neophytadiene (-1.17) is the least permeant compound.

Conclusion

In the research and development of phytocompounds, the tiny molecules derived from plants which alter metabolism are revealed as therapeutic agents. In the present study, we used the Swiss ADME web tool to evaluate hit molecules present in *C. gigantea* and *S. kunthiana.* This research demonstrated that the high-throughput screening using molecular docking and ADMET evaluation resulted in the verdict that neophytadiene, Phytol 9,12,15-Octadecatrienoic acid, (Z, Z, Z) revealed the best larvicidal, ovicidal, and repellent activity against *Cx. quinquefasciatus*. The lethal activity tests showed that they are in good agreement with the theoretically predicted binding of the active compound to the enzymes. Thus, in view of all the data reported in this study, seven compounds have a good larvicide and repellent activity. Finally, the outcomes confirm the use of phytocompounds of *C. gigantea* and *S. kunthiana* for mosquito control suggests that this plant might be a prospective resource for advancing a new mosquito controlling agent.

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