A search for mosquito larvicidal compounds by blocking the sterol carrying protein, AeSCP-2, through computational screening and docking strategies

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ABSRACT

Background: Sterol is a very vital compound for most of the insects and mosquitoes to complete their life cycle. Unfortunately mosquitoes cannot synthesize the sterol, it depends on mammals for the same. Mosquitoes take the sterol from the plant decays during their larval stage in the form of phytosterol, which is then converted to cholesterol for further growth and reproduction. This conversion occurs with the help of the sterol carrier protein 2(SCP2). **Methods:** Mosquito populations are controlled by plant-based inhibitors, which inhibit sterol carrier protein (SCPI-Sterol carrier protein inhibitor) activity. In this article, we explain the methods of inhibiting Aedes aegypti SCP2 by insilico methods including natural inhibitor selection and filtrations by virtual screening and interaction studies. **Results:** In this study protein-ligand interactions were carried out with various phytochemicals, as a result of virtual screening Alpha-mangostin and Panthenol were found to be good analogs, and were allowed to dock with the mosquito cholesterol carrier protein AeSCP-2. **Conclusion:** Computational selections of SCPIs are highly reliable and novel methods for discovering new and more effective compounds to control mosquitoes.

Key words: Mosquito sterol carrier protein, phytochemicals, computational screening, docking, SCPI.

INTRODUCTION

Worldwide, mosquitoes are notorious for spreading malaria, dengue, Japanese encephalitis, yellow fever, and west Nile Virus, and their number is increasing day by day. The World Health Organization (WHO) has estimated that there are nearly 300 million cases of mosquito-borne diseases annually. Malaria is the biggest killer, which claims a million lives a year.

The two main approaches to mosquito control are genetic and chemical. In the genetic approach, researchers are working on several methods to modify the mosquito's genetic pathways, so it cannot transmit diseases, but it can still take a blood meal. The problem in this approach is uncertainties about releasing genetically modified organisms into the environment. Therefore, a more

fine-tuned chemical approach is more practical; only one compound is selected, which works for a short period, and targets a single insect. These chemicals must be specific pesticides to kill only the mosquitoes, with low residue time, and they must not go down the same road as DDT. Looking at blocking of target proteins in insect (mosquito) physiology and the development and finding of potential inhibitions for them should be a promising approach in that direction.

Sterols are ubiquitous among eukaryotic organisms and serve both as bulk membrane lipid components and as precursors for additional metabolites such as mammalian steroid hormones, plant-based steroid hormones, and insect ecdysteriods,[1] The major sterols of plants and fungi contain alkyl substitutions at carbon 24, which is absent in cholesterol, the dominant sterol of virtually all animals.[1] Cholesterol, a hydrophobic, sticky substance accumulates on the lining of human arteries is an important component of the cell membrane, in vertebrates and invertebrates. In mosquitoes, it is vital for growth, development, and egg production, as unlike humans, mosquitoes cannot synthesize cholesterol. They must obtain it from the

decomposed plants they eat while in the larval stage, when living in shallow water. Plants make phytosterol, which is converted to cholesterol in the mosquito's gut. In order to transport it in a liquid medium, such as blood or cell fluids, the organisms must have a way to shield it from the watery environment through which it moves, which is studied typically in a carrier protein SCP-2.

Homology of SCP has been found across the animal kingdom, including insects. The latter are particularly interesting, because insects lose a number of key enzymes in the cholesterol biosynthesis pathways,[2] which results in a complete dependence on exogenous sources of cholesterol food synthesis for its steroid derivatives.[3,4] Hence, SCP-2 demands that insects must have mechanisms for uptake, transport, and storage of cholesterol, which is necessary throughout their life cycle. Indeed insects have the tendency to accumulate cholesterol in the body during the feeding stages, when their diet is richer in lipids.[5]

Intracellular transportation of cholesterol in insects must meet two important biological needs; first, the necessity to absorb free cholesterol for the constructions of cellular membranes, and later to provide cholesterol as precursors for steroid biosynthesis. These two pathways most likely utilize the same intracellular transport protein(s) to metabolize cholesterol. At this time, SCP-2 appears to be a good candidate as a participant for this task.[6]

Sterol Carrier Protein-2 (SCP-2) or the nonspecific lipid transfer protein was first isolated, as among the cholesterol transporters involved in cholesterol and lipid intracellular trafficking in vertebrates, it is a smaller protein with a molecular weight of 14 kilo Dalton. SCP-2 belongs to a family of proteins containing a sterol-binding domain (SCP-2 SCP-X, 17 B - Hydroxysteroid dehydrogenase type IV (HSD17B4), and stomatin).[7-9] The vertebrate SCP-2 SCP-X, HSD17B4 have a proximate localization sequence in the C-terminus, targeting these proteins to the peroxiosome.[7,8] The vertebrates' SCP-2 is characterized as a non-specific lipid carrier protein, which has an affinity for different ligands in the order, Cholesterol >>> straight chain fatty acid> kinked chain fatty acid.[10]

SCP-X has been reported to have high levels of expression in the midgut of Drosophila embryos; however, only a 1.6 kb mRNA transcript arises from this transcript.[11] This differs from vertebrates, where the SCP–X/SCP-2 gene combination produces multiple transcripts. In the yellow fever mosquito, *Aedes aegypti*, an independent gene has been identified that is similar to vertebrate SCP-2 (AeSCP-2). This protein also has high levels of expression in the midgut of the larvae and high binding affinity to cholesterol.[11]

The mosquito SCP-2 (AeSCP-2) appears to represents a unique non-peroxisomal and low molecular weight protein in the SCP-2 gene family.[11,12] Similar to the vertebrate SCP-2, AeSCP-2 also binds to cholesterol[11] and fatty acids;[6] similarly, both the vertebrate SCP-2 and the AeSCP-2 increase cholesterol uptake in overexpressed cells.[13]

Because insects do not synthesize cholesterol,[14] it is hypothesized that they may be involved is shuttling cholesterol and dietary steroids from lysosomes, from which exogenous sterol enters the cell and is transported to the endoplasmic reticulum and mitochondria. After conversion of dietary sterols to cholesterol or cholesterol to 7-dehydrocholesterold, in the endoplasmic reticulum, SCP-2 may also be involved in the transfer of cholesterol to the mitochondria, for steroid biosynthesis. The lack of a paroxysmal localization sequence in the C-terminus of AeSCP-2,[11] indicates that AeSCP-2 may be involved in the absorption and trafficking of cholesterol.

However, AeSCP-2 differs from the vertebrate SCP-2 in several aspects. In both cultured *A. aegypti* cells and in the larval midgut, AeSCP-2 localizes mostly in the cytosol, which is consistent with the fact that AeSCP-2 lacks the C-terminal peroxisome targeting sequence.[12] The coordination site for a ligand in AeSCP-2 is different from the vertebrate SCP-2, wherein, the hydrophobic moieties of these ligands are oriented at opposite ends of the protein.[6] AeSCP-2 seems to be a vital gene for the survival and development of mosquitoes, whereas, the vertebrate SCP-2 is not essential for its survival and fertility.[15] Knockdown of the AeSCP-2 expression in mosquito larvae leads to a high mortality rate in the emerging adults, and silencing of the AeSCP-2 in adults lowers the fertility.[16] Targeting cholesterol metabolism for the development of growth regulators in new insects, to control the insect population, is one of the goals of insect diseases causing vector management. Inhibitors are useful tools for elucidating the mode of action and molecular mechanisms of a functional protein. Searching for inhibitors of AeSCP-2 is a way to identify a chemical that could be used in mosquito control. If we block the carrier protein AeSCP-2, it would disrupt the uptake of cholesterol by the mosquito larvae and cause death.[17] A chemical library of 16000 compounds has been screened and 57 compounds that inhibit the cholesterol-binding capacity of SCP-2 have been found. Among them, the top five most viable compounds have been found to be effective in very small concentrations of about 10 ppm. In a similar manner, screening of phytochemicals obtained from herbal extracts have been reported earlier to possess larvicidal properties, which could be an alternate method to find out the viable inhibitor compounds that would disrupt the uptake of cholesterol by mosquito larvae.[18-24] As

mosquitoes and many other insects have become resistant to pesticides, heavy and frequent applications are required leading to problems of toxic residues contaminating the environment and adversely affecting non-target organisms. This dictates the need to develop safe, less expensive, and preferably locally available materials for mosquito vector control, and plant-based products are such potential tools. These products are the compounds that have evolved in plants for defense against phytophagous insects. Modern researchers have the technology to exploit the toxic properties of these compounds and use them against organisms that were never originally indented, in normal, vector diseases of modern man.

The present study is an attempt in the direction of compounds obtained from herbal extracts,[18-24] whose larvicidal activity was subjected to computational screening, to find out the potential phytochemicals that could block this carrier protein and thereby discover a new and more effective compound to control mosquitoes.

MATERIALS AND METHODS

The three dimensional crystal structure of the sterol carrier protein of *Aedes aegypti* (AeSCP-2) was obtained from the Protein data bank (PDB) (www.rcsb.org/pdb/). (PDB ID: 1PZ4) The coordinate file of AeSCP-2 was obtained by the molecular visualization viewer, the SPDB viewer (www.expasy.org/spdbv/). Amino acids in an active site of AeSCP-2 were from SER-18 to HIS-28[6], and it was confirmed with the help of binding pocket detection server tools such as pocket finder and Q-site finder (www. modelling.leeds.ac.uk/qsitefinder). The predicted binding sites, based on the binding energy, and 17 amino acids make up this binding cavity. The predicted ligand binding site residues are listed in Table 1.

Table 1
Selection of chemical compounds

The criteria set for chemical compound selection based on IC50 value, insecticidal activity, and medicinal values, are referred in various literatures and books. The Pubchem

database was used to retrieve the chemical compounds in the form of a SMILES notation (Simplified Molecular Input Line Entry Specification). The Pubchem database was used for retrieving the selected 133 phytochemical molecules. The selected chemical structures are generated from the SMILES notation by using the Chemsketch Software (www.acdlabs.com). After successfully building the structures, geometry optimization and energy minimization were done. The energy minimization process was carried out for 100 cycles, using the chimera software.

Creation of the lead database and virtual screening of ligands

The lead database for the selected compounds were built using Vega ZZ, and Screening was done for the 133 selected phytochemicals using Argus Lab. High-performance computing was used to analyze large datasets of chemical compounds, in order to identify possible drug candidates from the selected chemical entities. Screening was done by using the Argus Lab software for protein structure (1PZ4) of AeSCP-2. Molecules were taken from the top ranked ligand and interaction studies carried out in Auto dock 4.0

Protein – Ligand docking
(I) Protein Preparation

Autodock 4.0 is used for the docking process. The initial step for protein preparation is adding of polar hydrogens to the target protein AeSCP-2. Next the appropriate partial atomic charges are assigned. The charged protein is converted to the 'PDBQ' format so that Autogrid can read it. It is noted that in most modeling systems, polar hydrogens are added in a default orientation, assuming that each new torsion angle was 0° or 180°. Without some form of refinement, this will lead to spurious locations of the hydrogen-bonds. One option is that the hydrogens are relaxed and a molecular mechanics minimization is performed on the structure. Another one is that a program like "pol_h" is used, where the default-added polar hydrogen structure is taken as the input. Favorable locations for each movable proton are sampled and the best position of each is selected. This 'intelligent' placement of movable polar hydrogen would be particularly important for tyrosine, serine, and threonine amino acids.

(II) Ligand Preparation

Initially the hydrogens were added to all the atoms in the ligand and it was ensured that their valences were completed. This was done using ADT, a molecular docking package. It was ensured that the atom types were correct before adding the hydrogens. Depending on whether charged or neutral carboxylates and amides were desired, the PH was specified automatically. Next, the partial atomic charges were assigned to the ligand molecule. These charges were written in 'PDBQ' format, which had columns similar

Table 1: Binding site amino acids and its structural topology of AeSCP-2(1PZ4)	
Amino acids in the binding pocket	Binding site amino acids in the Structural unit of AeSCP2
VAL-8,PHE-9,ILE-12,ARG15 & LEU-16	Alpha -Helix
SER-18,ILE-19,ASP-20,ARG-24, GLN-25 & VAL-26	Ist Loop
TYR-30 & PHE-32	B-Sheet-I
MET-46 & LEU48	B-Sheet-II
LEU-64 & MET-66	B-Sheet-III

to a Brookhaven PDB format, but with an added column for partial atomic charges.

(III) Setting and Running of the Auto grid

The pre-calculated grid maps, one for each atom type present in the ligand being docked, were required for the Autodock to make the docking calculations extremely fast. These maps were calculated by the Autogrid. A grid map was created with a three-dimensional lattice of regularly spaced points, surrounding (either entirely or partially) and centered on the active site of the macromolecule, that is, 17 amino acids of AeSCP-2. Typical grid point spacing varied from 0.2Å to 1.0Å, although the default was 0.375Å (roughly a quarter of the length of a carbon–carbon single bond). The potential energy of a 'probe' atom or functional group was due to all the atoms in the macromolecule being stored in each point within the grid map. An input grid parameter file, which usually had the extension ".gpf", was required for the Autogrid. The maximum and minimum energies found during the grid calculations for AeSCP-2 were stored in the log file. With these important features of the Autogrid, it was set exactly on the active site of the AeSCP-2 (1PZ4) and the grid parameter file was written as a result of this process.

(IV) Running of the Auto Dock

The molecular docking was performed using a Genetic Algorithm — the Least Square (GA-LS) algorithm used in Auto dock 4.0. Once the grid maps had been prepared by the Autogird and the docking parameter file (dpf) was ready, the user could run an Auto Dock job. The docking results were viewed using 'get-docked'. It was called "lig. macro.dlg." and all the docked conformation outputs were viewed and analyzed. From the several poses of docking, the complex formed with least energy and with a stable conformation was taken.

RESULTS

From the result of docking with sterol carrier protein-2 (AeSCP-2), the best docked ligand molecules are selected based on docking energy and good interaction with the active site residues. Ten best conformations are retained with the energies and analyzed [Tables 2-5, Figure 1, 2].

DISCUSSION

Conventionally, mosquito control is carried out targeting
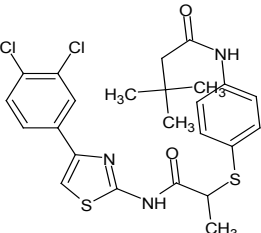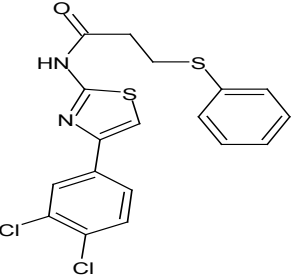
Table 2: Compounds selected for docking studies

Compound Name	Pubchem ID	Structure	M/w & Mol. Formula	H-Bond Donor/ Acceptor	Virtual Screening Score	Docking Energy (Kcal/mol)
Alpha-Mangostin	19712063		522.5102 $C_{24}H_{25}Cl_2N_3O_2S_2$	HBD-2 HBA-3	-12.5144	-13.65
Panthenol	4171189		409.35256 C18H14Cl2N2OS2	HBD-1 HBA-2	-12.3594	-9.23

Table 3: Ten best conformations of Alpha-mangostin and panthenol against AeSCP-2 and its corresponding binding energies

Inhibitor Compound Name	Binding Energy (Kcal/mol)									
	Conf 1	Conf 2	Conf 3	Conf 4	Conf 5	Conf 6	Conf 7	Conf 8	Conf 9	Conf 10
Alpha-mangostin	-2.53	-3.79	-3.49	-3.10	-3.78	-2.63	-3.11	-3.71	-2.00	-3.72
Panthenol	-2.95	-2.88	-2.74	-2.29	-2.03	-2.30	-1.97	-1.55	-1.02	0.96

either adults or immature larvae. Use of insecticides of chemical origin is the main stage in controlling the larval population of mosquito. However, continuous use of chemicals toward larval killing contaminates

Table 4: AeSCP2 – Ligands docking H-bonding interaction table

Conformation	Inhibitor 1: Alpha Mangostin		Distance (Å)
	AeSCP2		
	Residue	Atom	
1	Ala22	HN O	2.91
2	Ser18	NH O	3.20
3	Tyr30	O H	2.49
4	Gln35	H O	2.39
5	Leu53	O H	2.03
6	Glu55	HN O	2.73
7(I)	Leu53	HN O	2.93
7(II)	Leu53	O H	3.01
8	No H- Bonds are formed		
9	Lys31	H O	2.20
9(II)	Thr63	O H	3.02
10	TYR30	O H	2.74

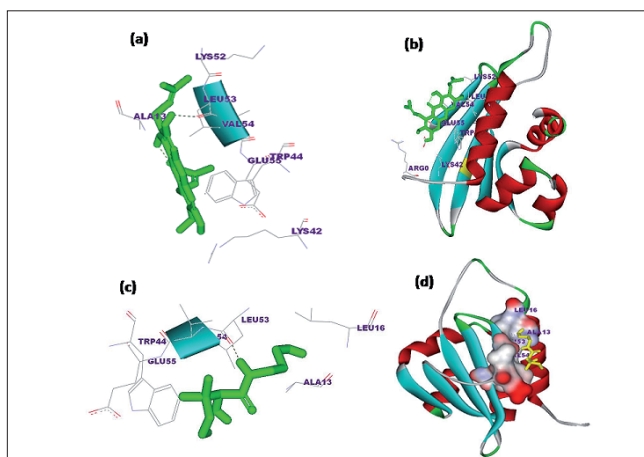| Conformation	Inhibitor 2: Panthenol		Panthenol	Distance (Å)
	AeSCP2			
	Residue	Atom		
1	Leu53	O	H	1.95
2	Leu53	O	H	1.95
3	Leu53	O	H	2.08
4	No H-Bonds are formed			
5	Leu53	O	H	2.03
6(I)	Glu55	HN	O	2.15
6(II)	Leu53	O	H	1.85
7(I)	Leu53	HN	O	1.96
7(II)	Leu53	O	H	2.16
7(III)	Glu55	HN	O	1.96
8	Leu53	O	H	2.09
9(I)	Lys31	HZ2	O	2.08
9(II)	Ala60	O	H	1.85
10	No H-Bonds are formed			

Figure 1: Result of docked conformation of Alpha-mangostin and Panthenol. (a) and (b) Fifth and sixth best conformations of Alpha mangostin, with the binding energies of -3.78 and – 2.63 kcal/mol, respectively. (c) and (d) Eighth and ninth conformations of panthenol with binding energies of -2.03 and – 1.97 kcal/mol, respectively.
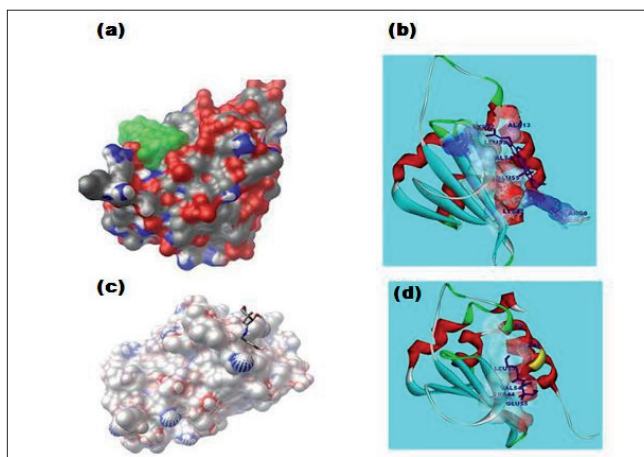

Figure 2: (a) Electrostatic interaction of the AeSCP-2 – Alpha mangostin complex. (b) AeSCP-2-Alpha mangostin interactions in ribbon view (c) Electrostatic interaction of AeSCP-2 – Panthenol complex (d) AeSCP-2 – Panthenol interactions in ribbon view

Table 5: Final docked energies of the selected compounds

(a) Docked conformation of Alpha-Mangostin

```
MODEL    22
USER   Run = 22
USER   Cluster Rank = 1
USER    Number of conformations in this cluster = 1
USER
USER   RMSD from reference structure      = 213.739 A
USER
USER   Estimated Free Energy of Binding    =   -8.16 kcal/mol [= (1) + (3)]
USER   Estimated Inhibition Constant, Ki   =+1.05e-06[Temperature = 298.15 K]
USER
USER   Final Docked Energy            = -13.65 kcal/mol [= (1) + (2)]
USER
USER   (1) Final Intermolecular Energy    = -13.45 kcal/mol
USER   (2) Final Internal Energy of Ligand =   -0.20 kcal/mol
USER   (3) Torsional Free Energy        =  +5.29 kcal/mol
USER
USER
USER   DPF = ptn.dpf
USER   NEWDPF move   lig.pdbq
USER   NEWDPF about   2.875200 -1.618300 -5.191300
USER   NEWDPF tran0   106.188101 83.347625 169.606940
USER   NEWDPF quat0   -0.746322 -0.282636 0.602594 5625.071964
USER   NEWDPF ndihe   14
USER   NEWDPF dihe0   27.88 71.76 -46.97 82.15 -72.75 70.60 -19.46 -59.78
         111.66 -1.09 -41.02 57.61 -72.08 116.32
```

(b) Docked conformation of Panthenol

```
MODEL    60
USER   Run = 60
USER   Cluster Rank = 1
USER    Number of conformations in this cluster = 1
USER
USER   RMSD from reference structure      = 34.505 A
USER
USER   Estimated Free Energy of Binding    =  -8.17 kcal/mol  [=(1)+(3)]
USER   Estimated Inhibition Constant, Ki =+1.03e-06[Temperature = 298.15 K]
USER
USER   Final Docked Energy            =  -9.23 kcal/mol  [=(1)+(2)]
USER
USER   (1) Final Intermolecular Energy    = -10.97 kcal/mol
USER   (2) Final Internal Energy of Ligand =   +1.74 kcal/mol
USER   (3) Torsional Free Energy        =  +2.80 kcal/mol
USER
USER
USER   DPF = target.dpf
USER   NEWDPF move   ligand.pdbq
USER   NEWDPF about   16.464800 -10.331200 -0.169100
USER   NEWDPF tran0   35.035589 8.790575 24.843612
USER   NEWDPF quat0   0.298628 -0.935809 0.187305 -40535.517000
USER   NEWDPF ndihe   8
USER    NEWDPF  dihe0  54.10 98.68 -103.62 94.74 -121.31 -72.63 109.96 -  68.54
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the environment. This warrants alternate eco-friendly products for controlling the immature larvae of the vector mosquitoes. Cholesterol uptake is the important step for larval population. Cholesterol conversion/uptake are carried out in the presence of the carrier protein AeSCP-2.

To block the carrier protein, several compounds were screened. In the present study the phytochemicals, namely, Alpha-mangostin and Panthenol were found to be good analogs, and were allowed to dock with the mosquito cholesterol carrier protein AeSCP-2. Earlier several AeSCP-2 inhibitors (SCPIs) were identified. SCPIs belonged to several chemotypes of hydrophobic compounds. Based on the inhibitory effect of SCPIs on AeSCP-2 cholesterol binding *in vitro* and cholesterol uptake in cultured insect cell's it was assumed that SCPIs showed high larvicidal activities in the yellow fever mosquito, *Aedes aegypti*, and in the tobacco horn worm, *Manduca sexta*, even though SCPIs had very low cytotoxicity in cultured mouse cells.[17] Kim *et al*. reported that five SCPIs, namely, N-(4{4-(3-4-dihidrophenyl)-1,3-thiazol-2-yl]amino}phenyl)acetamide hydro bromide, 8-chloro-2-(3-methoxyphenyl)-4,5-dihydroisothiazolo{5,4-c] quinonoline-1 (2H_triune,3-(4-bromophenyl)-5-methoxy-7-nitro-4H-1,2,4-benzoxzdiazine,4,4,8-trimethyl-5-(3-emthylbutanoyl)-4,5dihydro-]H-[1,2]dithiolo[3,4-c] quinoline-1-thione3-bromo-N-{2-[4-chloro-2-nitrophenyl) amino]ethyl}-4-ethoxy benzamide, were compared with cholesterol for AesCP-2 and found that these AeSCP-2 specific inhibitors exhibited physiological effects on cholesterol metabolism in cultured insect cells, which were similar to the effects of AeSCP-2 knockdown. They also reported the bio efficacy of these chemicals to SCPIs. Even these types of chemical analogs were found to be toxic and lethal to the other species. To avoid this condition, we used plant-based chemicals, extracted from plants, for the initial virtual screening and interaction studies using the bioinformatics approach.

From this study we found two potential inhibitors namely alpha-mangostin and panthenol. They had effective interactions in the binding site of AeSCP-2. In the next level we can observe the feasibility of using these inhibitor compounds in the fields.

CONCLUSION

As the identified ligands of phytochemical origin, it may be presumed that these are safer in the environment as well. The chemical interaction between selected ligands (both alpha mangostin and panthenol) and the target protein (AeSCP-2) has been found to be good and has the best binding energy and interaction scores. This study will help understand how the target protein is arrested by the ligands and inhibits the sterol carrier pathway. Further research is needed for refinement, for enriching the activity of the ligands and attacking the AeSCP-2, especially in the watery environment, and also to determine the dosages of safety levels, to explore this promising avenue for mosquito control and ensure the healthy state of humans.

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