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In silico screening of phytocompound inhibitors against sterol carrier protein (AeSCP-2) in Aedes aegypti

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Abstract

The major component for insect's growth is cholesterol, which is regulated by sterol carrier protein 2 (SCP-2). Naturally, the plants contain numerous active compounds, acting as inhibitors for proteins carrying sterols (AeSCP-2). Therefore, in the present study overall, 21 phytochemicals were selected from previously reported plants with insecticidal activity, i.e., *Citrullus colocynthis* (L.), *Cannabis indica* (L.) and *Artemisia argyi* (L.), and were utilized for dock (*in silico*) with the mosquito AeSCP-2. Among all the compounds, 1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl (methyl triphenyl acetate), 2-Isopropenyl-2,3-dihydrofuro[3,2-g] chromen-7-one and 4-Nitro-4'-chlorodiphenylsulfoxide yielded results with the lowest docking energy, i.e., -13.7362, -11.993 and -11.3161 Kcal/Mol, respectively. Among all the three compounds, methyl triphenyl acetate displayed the lowest binding energy score than others. It is also a major phytocompound found in *Artemisia argyi* extracts, and this plant might have displayed insecticidal activity due to the presence of methyl triphenyl acetate, which, based on the *in silico* studies, act as an AeSCP-2 inhibitor.

Keywords: cholesterol, sterol carrier protein, AeSCP-2, methyl triphenyl acetate and mosquitoes

Introduction

Dengue fever is a virus spread by Aedes mosquitos and is one of the most common viral diseases. Dengue fever affects approximately 3.9 billion people in 129 countries, with an estimated 96 million symptomatic cases and 40,000 deaths per year[1]. Controlling the mosquito population is critical for preventing the spread of mosquito-borne diseases and improving the environment and public health. Synthetic insecticides such as organochlorine and organophosphate compounds are widely used to control mosquitoes. Human, operational, technical, ecological, and economic factors have rendered this management ineffective. Many of these old synthetic insecticides have been restricted in recent years. For example, the lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, resistance development, harmful effects on human health and other non-target populations, non-biodegradable nature, and increasing global insecticide resistance contribute to these factors[2,3].

Most insects lack essential enzymes for cholesterol biosynthesis, rendering them incapable of producing cholesterol from scratch [6]. As a result, they rely on external sources for cholesterol, a building block for bio compounds like moulting hormones7. Sterol carrier protein 2 (SCP-2) is a cytosolic protein involved in cholesterol binding and transport and is particularly important in the mosquito's acquisition of dietary cholesterol [1, 7], Dyer *et al*., 2008). SCP-2 expression is inhibited, resulting in slower larval growth and lower adult fertility [7, 8]. As a result, inhibiting any critical steps for cholesterol uptake will result in the insect's death. This strategy can be used to combat vector-borne diseases as a vector control method.

Molecular docking is an *in-silico* technique for determining biding poses and free energy values by estimating the strength of the protein-ligand interaction[4]. Docking is a term that refers to the non-covalent interactions that occur when a ligand binds to a receptor. As a part of new drug development, it is frequently used to investigate ligand recognition on the target molecule. Kumalo and colleagues (2015) developed a formalized formalized formalized formalized formalized [5]. Various natural products derived from plants have been tested for their ability to control *A. egypti* and have demonstrated insecticidal, larvicidal, and repellent properties.

Additionally, many secondary metabolites produced by plants, such as essential oils, alkaloids, and phenolics, have been reported to possess a variety of pharmaceutical and insecticidal properties in the past [9]. Recently, stated that the *Citrullus colocynthis* (L.), *Cannabis indica* (L.) and *Artemisia argyi* (L.) extracts showing insecticidal activity against *Brevicoryne brassicae* [15]. Hence, the current study aims to determine the best insecticidal phytocompound from the selected 21 phytocompounds through*in silico* docking studies by evaluating their mosquito sterol carrier protein (AeSCP-2) inhibitory properties.

Materials and methods

Insilico study

To obtain the three-dimensional crystal structure of the sterol carrier protein of the mosquito Aedes aegypti (AeSCP-2), the researchers used the Protein Data Bank (PDB) (website: http://www.rcsb.org/pdb/) and the PDB ID: 1PZ4 (http://www.rcsb.org/pdb/). The coordinate file for AeSCP-2 was obtained using the SPDB viewer (http://www.expasy.org/spdbv/), a molecular visualization

viewer (http://www.expasy.org/spdbv/). An active site of AeSCP-2 contained amino acids ranging from SER-18 to HIS-28, as determined by binding pocket detection server tools such as pocket finder and Q-site finder (www.modelling.leeds.ac.uk/qsitefinder) and confirmed by crystallographic analysis. The binding cavity comprises 17 amino acids and the binding sites (which were predicted based on the binding energy) (Table 1).

Table 1: Binding site amino acids and its structural topology of AeSCP-2 (PDB: 1PZ4)

Amino acid in the binding pocket		Binding site amino acids in structural unit of AeSCP2
VAL-8, PHE-9, ARG15 & LEU-16	:	Alpha – Helix
SER-18, ILE-19, ASP-20, ARG-24, GLN-25 &VAL-26	:	IstLoop
TYR-30 & PHE-32	:	B-Sheet-I
MET-46 & LEU48	:	B-Sheet-II
LEU-64 & MET-66	:	B-Sheet-III

Selected compounds

It was necessary to use the Pubchem database to retrieve the phytocompounds chosen (Table 2). A chemical structure was Chemsketch Software was used to generate the SMILES notation, which was then used to generate the other chemical structures (www.acdlabs.com). After the structures were successfully constructed, the geometry optimization and energy minimization processes were carried out. Using the chimera software, the process of energy minimization was repeated 100 times for each cycle.

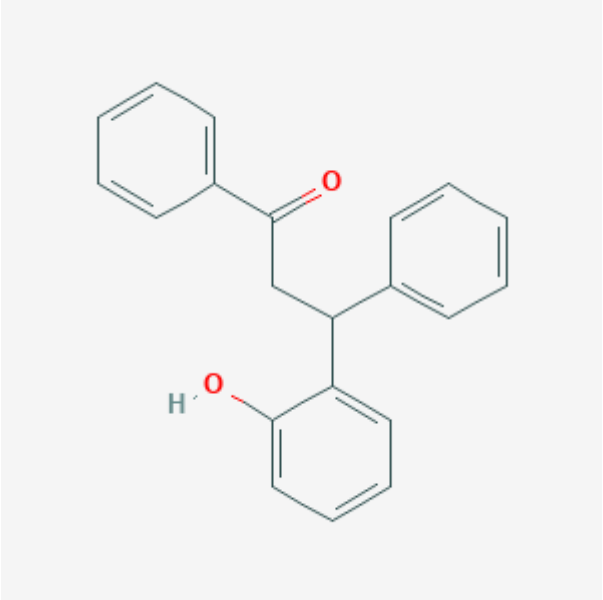

Fig 1: Molecular structure of 1-Propanone, 3-(2-hydroxyphenyl)-1, 3-diphenyl

Table 2: The properties of selected phytocompounds

S.No	Compounfd	Pubchem id	Chemical formula	Molecular wait	Hydrogen donor and acceptor	SMILES
1	1,2-Ethanediol, diformate	12376	C4H6O4	118.09	0,4	C(COC=O)OC=O
2	1,3,4-Trimethoxydibenzofuran	71409659	C15H14O4	258.27	0,4	COC1=CC(=C(C2=C1C3=CC=CC=C3O2)OC)OC
3	1,5-Heptadien-4-ol, 3,3,6-trimethyl-	100197	C10H18O	154.25	1,1	CC(=CC(C(C)(C)C=C)O)C
4	1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl-	610256	C21H18O2	302.4	1,2	C1=CC=C(C=C1)C(CC(=O)C2=CC=CC=C2)C3=CC=CC=C3O
5	2,5-Cyclohexadien-1-one, 2,5-dimethyl-4-[(2,4,5-trimethylphenyl) imino]-	620100	C17H19NO	253.34	0,2	CC1=CC(=C(C=C1C)N=C2C=C(C(=O)C=C2C)C)C
6	2,11-Dimethyl-2,3,4,5,6,7-hexahydro-1H-2-benzazonine	608948	C14H21N	203.32	0,1	CC1=C2CN(CCCCCC2=CC=C1)C
7	2-Ethylacridine	610161	C15H13N	207.27	0,1	CCC1=CC2=CC3=CC=CC=C3N=C2C=C1
8	2-Isopropenyl-2,3-dihydrofuro[3,2-g]chromen-7-one	611672	C14H12O3	228.24	0,3	CC(=C)C1CC2=C(O1)C=C3C(=C2)C=CC(=O)O3
9	3-Methoxybenzyl alcohol	81437	C8H10O2	138.16	1,2	COC1=CC=CC(=C1)CO
10	4-Nitro-4'-chlorodiphenylsulfoxide	624554	C12H8ClNO3S	281.72	0,4	C1=CC(=CC=C1[N+](=O)[O-])S(=O)C2=CC=C(C=C2)Cl
11	5H-Naphtho[1,8-bc]thiophen-5-one,3,4-dihydro-2-methyl	608780	C12H10OS	202.27	0,2	CC1=C2CCC(=O)C3=C2C(=CC=C3)S1
12	5-Methyl-2-phenylindolizine 1	15133523	C17H15NO	249.31	0,1	CC1=CC=CC2=C(C(=CN12)C3=CC=CC=C3)C(=O)C
13	5-Methyl-2-phenylindolizine	610180	C15H13N	207.27	0,0	CC1=CC=CC2=CC(=CN12)C3=CC=CC=C3
14	6H-Dibenzo[b,d]pyran-1-ol, 6,6,9-trimethyl-3-propyl- (Cannabivarin)	622545	C19H22O2	282.4	1,2	CCCC1=CC(=C2C(=C1)OC(C3=C2C=C(C=C3)C)(C)C)O
15	a-Bisabolola-Bisabolol, alpha-Bisabolol	10586	C15H26O	222.37	1,1	CC1=CCC(CC1)C(C)(CCC=C(C)C)O
16	Acridine	9215	C13H9N	179.22	0,1	C1=CC=C2C(=C1)C=C3C=CC=CC3=N2
17	Benzenesulfonic acid, 4-nitro	95081	C8H9NO5S	231.23	0,5	CCOS(=O)(=O)C1=CC=C(C=C1)[N+](=O)[O-]
18	Caryophyllene oxide	1742210	C15H24O	220.35	0,1	CC1(CC2C1CCC3(C(O3)CCC2=C)C)C
19	Cyclobarbital	5838	C12H16N2O3	236.27	2,3	CCC1(C(=O)NC(=O)NC1=O)C2=CCCCC2

20	Dronabinol	16078	C21H30O2	314.5	1,2	CCCCCC1=CC(=C2C3C=C(CCC3C(OC2=C1)(C)C)C)O
21	N-Isopropyl-3-phenylpropanamide	541827	C12H17NO	191.27	1,1	CC(C)NC(=O)CCC1=CC=CC=C1

Molecular docking using Auto dock 4.0

Auto dock 4.0 was used to study interactions between molecules from the top-ranked ligand.

Protein-Ligand docking

1. Protein Preparation

To complete the docking process, Auto dock 4.0 was utilized. AeSCP-2, the target protein, was treated with polar hydrogens in the first few steps of the protein preparation procedure. It was then necessary to assign the appropriate partial atomic charges. A modified PDBQ format was created for Autogrid to be able to recognize the charged protein. Polar hydrogens are typically introduced in the default orientation in most modelling systems, assuming that each new torsion angle is between 0 and 180 degrees. It would result in erroneous hydrogen bond locations if this type of refinement were not used. After relaxing the hydrogens, the structures were minimized molecular mechanics, one of the pursued options. Another method would be to use a program such as "pol h," which was used to carry out the same task. As the input, the default polar hydrogen structure is added. The best locations have been selected for each moving proton, and the best position has been chosen for each proton. It would be critical to have an "intelligent" placement for the moving polar hydrogen in amino acids like tyrosine, serine, and threonine.

2. Ligand Preparations

To begin, hydrogens were added to each ligand's atoms, with care taken to ensure that their valences were fully completed before moving on to the next step. This was accomplished through the use of ADT, a molecular docking package. Before adding the hydrogens, it was necessary to ensure that the atom types were correct. It was thus possible to specify the PH of the carboxylates and amides automatically based on whether charged or neutral carboxylates and amides were wanted. The ligand molecule's structure was then used to assign partial atomic charges to the ligand molecule. They were all written in the 'PDBQ' format, which featured columns identical to those present in the Brookhaven PDB format, but with an additional column for incomplete atomic charges, as was the case with the Brookhaven PDB format.

3. Configuration and operation of the Auto grid

To speed up the docking calculations, pre-calculated grid maps for each atom type in the ligand were required. Using Autogrid, these maps were created. Using a three-dimensional lattice of regularly spaced points, it was possible to create a grid map that surrounded (either completely or partially) and was centred on the active site of the macromolecule, which comprises 17 amino acids in AeSCP-2. It was customary to have grid points spaced anywhere between 0.25 and 1.00 inches apart, with 0.375 inches being the default. In this case, all of the atoms in the macromolecule were stored at each point on a grid map, which resulted in potential energy for the 'probe' atom or functional group. The "Autogrid" required the use of an input grid parameter file, which was typically saved with the extension ".gpf." The log file was used to record maximum and minimum energies discovered during AeSCP-2 grid calculations. Due to the Autogrid's critical characteristics, it was placed precisely on the AeSCP-2 active site (1PZ4), resulting in the grid parameter file.

4. Running of the Auto Dock

A genetic algorithm — the GA-LS (Last Square) algorithm — has been used to dock the molecules, which was implemented in Auto dock 4.0. After the Autogird has prepared the grid maps and the docking parameter file (dpf) was created, Auto Dock could be started. The docking results were viewed with the help of the 'getdocked' command. It was given the name "lig.macro.dlg." It allowed users to view and analyze all of the docked conformation outputs. An energy-efficient and stable complex were chosen from various docking poses.

Result

Among all the compounds screened for docking against carrier sterol protein (PDB ID: 1PZ4) 1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl or methyl triphenyl acetate, 2-Isopropenyl-2,3-dihydrofuro[3,2-g] chromen-7-one and 4-Nitro-4'-chlorodiphenylsulfoxide yielded results with the lowest docking energy, i.e., -13.7362, -11.993 and 11.3161 Kcal/Mol, respectively. Thus, among all the three compounds, 1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl- or methyl triphenyl acetate displayed the lowest binding energy score. 1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl- formed 1 hydrogen bond, 1 Pi-anion and 9 Pi-alkyl interactions with the active site region. The residue GLU 103 formed hydrogen of 2.38 Ǻ, whereas the other residues LYS 82, ALA 81, LEU 102, LEU 64, ILE 99, VAL 96, MET 85, and VAL 92 were involved in Pi-alkyl interactions in the active site. Figure A & B display the interactions between 1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl or methyl triphenyl acetate with the active site of the protein (Figure 2).

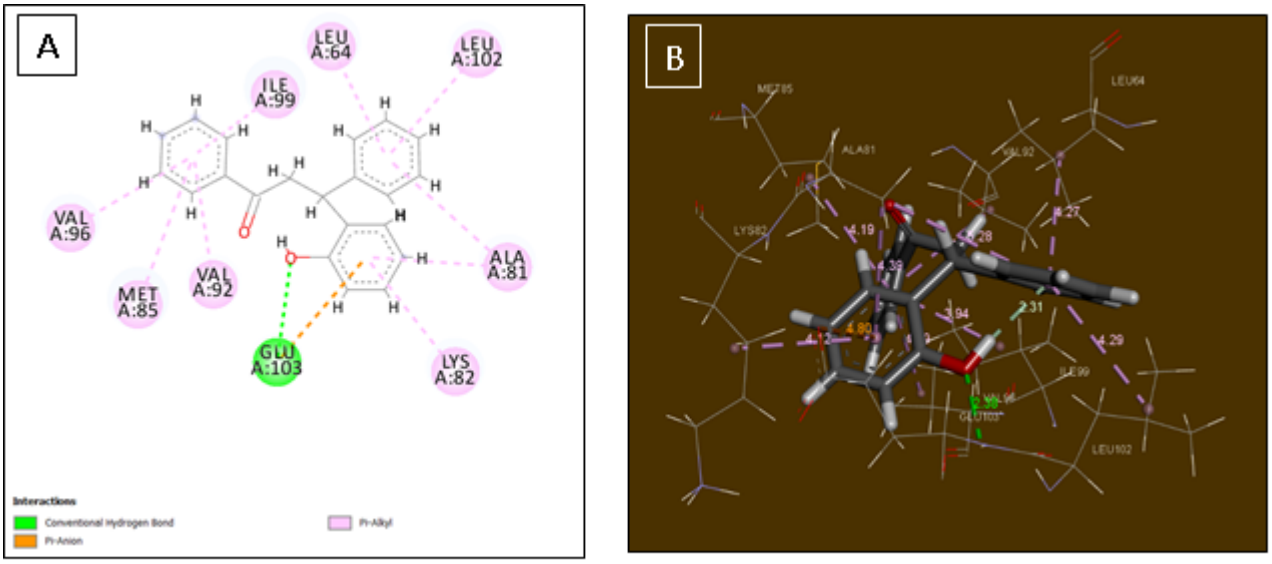

Fig 2: A) 2D image showing the interactions between the ligand 1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl and the protein active sites. B) 3D image of the interactions between the ligand 1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl and the protein active sites with bond lengths.

Discussion

Currently, mosquito larvae and pupae are mainly controlled using synthetic chemical insecticides. However, extended use of these pesticides has resulted in numerous issues, such as developing resistance, unwanted effects on non-target organisms, effects on wildlife, harm to human health and other adverse environmental effects [4]. Mosquito populations can also be controlled by plant-based inhibitors, which inhibits sterol carrier protein (SCPI-Sterol carrier protein inhibitor) activity. In this investigation, we explain the methods of inhibiting *Aedes aegypti* SCP2 by *in silico* methods, by screening natural phytocoumpounds that can act as possible inhibitors.

In insects, hormones that promote moltings, such as ecdysone and 20-hydroxyecdysone, are produced using cholesterol as a precursor to the hormone [11]. Ecdysteroids are the hormonal precursors to the insect moulting process. Ecdysteroids are also necessary for the growth and development of insects, as they aid in their growth and development [12, 13]. Ecdysteroids are secreted by insects during their juvenile phase, and the subsequent decline in the juvenile hormone (JH) to zero aids in the secretion of ecdysteroids, which in turn aids in the initiation of the next moult. Subsequently, the hormonal actions cause an increase in JH levels, resulting in a decrease in the secretion of the ecdysteroid hormone. These low ecdysteroid levels set off this episode of dysphoria [12, 13]. In the absence of adequate cholesterol levels, it will disrupt the normal hormone levels, and ecdysis, or the process of insect moulting, will not be triggered. This may result in growth disruptions, resulting in the insect's death at the larval stage without the opportunity to progress further in its life cycle [14]. It is necessary to have the carrier protein AeSCP-2 present for cholesterol conversion and uptake to occur [10]. Various researchers have tested several compounds to see if they can inhibit the carrier protein AeSCP-2 [16, 17].

In the present study, overall, 21 phytochemicals found in *Citrullus colocynthis* (L.), *Cannabis indica* (L.) and *Artemisia argyi* (L.) were utilized to dock (*in silico*) with the mosquito cholesterol carrier protein AeSCP-2. Among the 21 selected phytocompounds, methyl triphenyl acetate displayed the lowest binding energy score (-13.7362) and formed one hydrogen bond, 1 Pi-anion and 9 Pi-alkyl interactions with the active site region than other phytocompounds. Previously, they reported that the methyl triphenyl acetate is a major phyto compound in *Artemisia argyi* extracts, and this extract was showed insecticidal properties against cabbage aphids [15]. This result indicates that methyl triphenyl acetate is a major compound for the insecticidal property of *Artemisia argyi*, and it may kill the insects by inhibiting cholesterol uptake, causing metabolic and hormonal imbalance, and inhibits the synthesis of SCP-2.

Conclusion

The present results demonstrated that among all the 21 selected phytocompounds, methyl triphenyl acetate displayed the lowest binding energy score (-13.7362). As methyl triphenyl acetate is one of the major phytocompounds present in *Artemisia argyi*, the above results revealed that *Artemisia argyi* extract might have insecticidal activity due to its presence of methyl triphenyl acetate, which can inhibit the cholesterol metabolism in mosquitoes leading to its death. Further, it is non-toxic to humans and can be assumed to be eco-friendly. Thus, based on the present results, further study (*in vitro*) must be carried out on methyl triphenyl acetate for its long-term effect.

Conflict of interest

The authors have no conflicts of interest regarding this investigation.

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