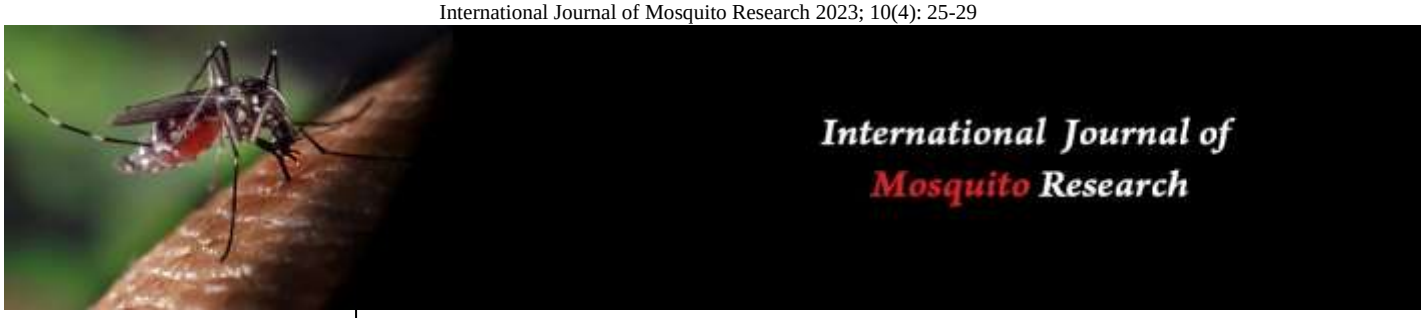


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# Identification of a plant derivative (*Hibiscus cannabinus*) for mosquito (*Anopheles darlingi*) control using *in silico* protein-protein docking techniques

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**Abstract**
*Hibiscus cannabinus,* often known as kenaf, is contain significant amount of bioactive compounds and acts as a natural antioxidant. The aim of this *in silico* investigation is to find out how the flower extract, *Sulfite oxidase* inhibits the mosquito (*Anopheles darlingi*) protein, pro-resilin, using 3D automated drug docking studies. In this methodology, we perform primary analysis on the amino acid sequences of pro-resilin and sulphite oxidase using NCBI GenPept and HDock server for performing drug docking analysis. The overall results clearly elucidate that the intramolecular dynamic interaction between Sulfite oxidase and the motif regions of Pro-resilin is very effective. This results in the downregulation of the expression of Pro-resilin which has been shown in 3D form. At present, research on mosquito control is of prime focus at global level. Our *in silico* research investigation is a safe initiative to control mosquitoes using flower extract as it is devoid of side effects.

**Keywords:** Anopheles darlingi, *Hibiscus cannabinus*, pro-resilin, protein docking

## Introduction
Since the late twentieth century, there has been a lot of research into the replacement of a disease vector or an agricultural pest with a more benign strain [1, 4]. At the moment, sterile insect methods and the development of transgenic mosquitoes with diminished Plasmodium competency are being applied in studies aimed at the management and replacement of Anopheles populations [5, 6]. However, given the stresses of shifting and altered habitats and surroundings, as well as influence imposed directly by humans through the application of insecticide and pesticides, replacement and genetic turnover events do occur naturally and may be very common. Microsatellites were used to detect such an occurrence in a recent, well-documented case involving the agricultural pest *Bemisia tabaci* (silverleaf whitefly) in Queensland, Australia. The most numerous subpopulation of the silverleaf whitefly was virtually fully replaced by a significantly less numerous one within a 3-month period between 2006 and 2007, while the causes of this replacement are unknown [10]. *Aedes triseriatus* was invaded and largely displaced in New Jersey over a 9-year period by *Aedes albopictus* and *Aedes japonicus*. *Ae. albopictus* and *Ae. japonicus* both had a doubling in abundance during this time, but *Ae. triseriatus* experienced a three-fold decline [11]. *Ae. triseriatus* is a known arboviral vector [12], but studies have shown that *Ae. albopictus* and Ae. japonicus are far more capable carriers of a variety of arboviruses, including chikungunya and dengue [13, 16]. Additionally, between August 2008 and March 2010, species replacement was seen in anophelines in the Brazilian Amazon (Amazonas state).
The catalytic molybdenum atom is positioned in the centre of a square-based pyramid in the Moco domain of SOX, where it coordinates five atomic ligands [17, 18, 19]. Sulphur atoms make up three of these ligands, and two of them come from the dithiolate moiety of the Moco scaffold. The SOX polypeptide chain's human Cys264, a highly conserved cysteine residue, provides the third sulphur [20]. The final two ligands are oxygen atoms, one of which is coordinated axially with the molybdenum atom and the other of which points in the direction

of the SOX active site. The latter is an example of the reactive oxoligand that is employed in the process of oxotransfer to create sulphate from sulfite [21]. The abstracted oxoligand is replaced by oxygen coordination of a water molecule during oxotransfer, which lowers the molybdenum atom's oxidation state from Mo (VI) to Mo (IV) [22, 23, 24, 25, 26, 27, 28]. The reductive half reaction of SOX is described by this.

## Methodology

**Protein sequence retrieval:** The target protein sequences, ACU33027.1 of sulfite oxidase from Hibiscus cannabinus and XP_049540068.1 of pro-resilin from *Anopheles darlingi* were retrieved from NCBI GenPept database 26 (https://www.ncbi.nlm.nih.gov/protein/). The 2D structure was converted into 3D structure using Discovery studio software in FASTA format in order to perform drug docking studies.

**Molecular Drug Docking:** The selected mosquito protein sequence and the predicted 3D structure of Malic acid were subject to drug docking studies in order to find out the binding efficiency of Sulfite oxidase with Pro-resilin of *Anopheles darlingi.* The molecular drug docking server, HDOCK server 27 (http://hdock.phys.hust.edu.cn/) was used for docking studies.

## Results and Discussion

The selected mosquito sps, *Anopheles darlingi* has pro-resilin whose amino acid sequence has 162 a (XP_049540068.1) The extract from *Hibiscus cannabinus* contains Sulfite oxidase whose amino acid length is 393 a (ACU33027.1) retrieved from NCBI database in FASTA format. (Fig: 1 and 2) Except for yeasts, all eukaryotes have sulfite oxidase in their mitochondria. Through the conversion of sulfite to sulphate and the transmission of the generated electrons to the electron transport chain via cytochrome c, oxidative phosphorylation is able to make ATP. The sulphate is eliminated after this final stage of the metabolism of substances containing sulphur. Sulfite oxidase is a metallo-enzyme that uses a heme group (in the case of mammals) and a molybdopterin cofactor. It is a cytochrome b5 and a member of the molybdenum oxotransferase enzyme superfamily, along with DMSO reductase, xanthine oxidase, and nitrite reductase .[28, 29, 30]

Elastomeric protein known as resilin is present in a variety of insects and other arthropods. It gives mechanically active organs and tissue a soft rubber-elasticity that, for instance, allows insects of many species to efficiently leap or rotate their wings. Torkel Weis-Fogh was the first to identify resilin in locust wing-hinges. The most effective elastic protein currently understood is resilin. Only 3% of the stored energy is wasted as heat, giving the isolated resilin from locust tendon a claimed 97% elastic efficiency [31].

The target protein of Pro-resilin from *Anopheles darlingi* is bound by the chemical structure, which is based on internal electrostatic force. (Fig: 3) Homologous complexes with a sequence identity of less than 30% with the test cases were disregarded throughout the HDOCK server evaluation. The unbound structural sequences were used as input for receptor and ligand as well as the protein-protein docking process. It was successfully determined throughout the evaluation that the projected binding mode had an allowable precision or better in accordance with the CAPRI criteria. Our findings are consistent with earlier research that has been established [32, 32, 33, 34, 35, 36, 37]. The larger negative value between sulphite oxidase and pro-resilin in our medication scores is 244.36 *kcal/mol* (Table 1).

Theoretically, a larger negative value denotes a good binding relationship between the medication and receptor. The binding interaction between pro-resilin and the Sulfite oxidase drug is seen in figure 4 and 5 at the hydrogen bonds. Using Discovery Studio Software, the results show the whole interaction together with the labels for the respective amino acids in a 3D format. The interaction between the acceptor and donor, specifically how sulphite oxidase suppresses the functional component of pro-resilin, is clearly seen in

The results of the molecular dynamic study revealed that the total length of the protein, pro-resilin of *Anopheles darlingi* was 162 aa (Insect cuticle protein –binding domain range (79-150). Within this length, various functional domains, such as, Moybdenum cofactor oxidoreductase, dimerisation (259-384) IPR005066 [38, 49] and Oxidoreductase, molybdopterin-binding domain (53-234) IPR000572 [40] are present. The binding interaction of Sulfite oxidase with pro-resilin takes place at the H-bond interacting amino acid positions (LYS:108,TRP:254,ARG:384,LYS:100,ARG:109,ARG:120, GLU:148,TYP:112,GLU:148,ASP:106.ASP:274,THR:104,TRP:117,SER:8,LYS:162,ILE:252.).

All of the studies show unequivocally that Sulfite oxidase binds to the pro-resilin's functional region, thereby, inhibiting it. Works similar to this have already been proved by us previously [41].

**Table 1:** Computational Drug docking summary of drug and receptor with the binding score along with units

| Drug | |
|---|---|
| Receptor | ACU33027.1 sulfite oxidase [*Hibiscus cannabinus*] |
| XP_049540068.1 pro-resilin [*Anopheles darlingi*] | -244.36 *kcal/mol* |

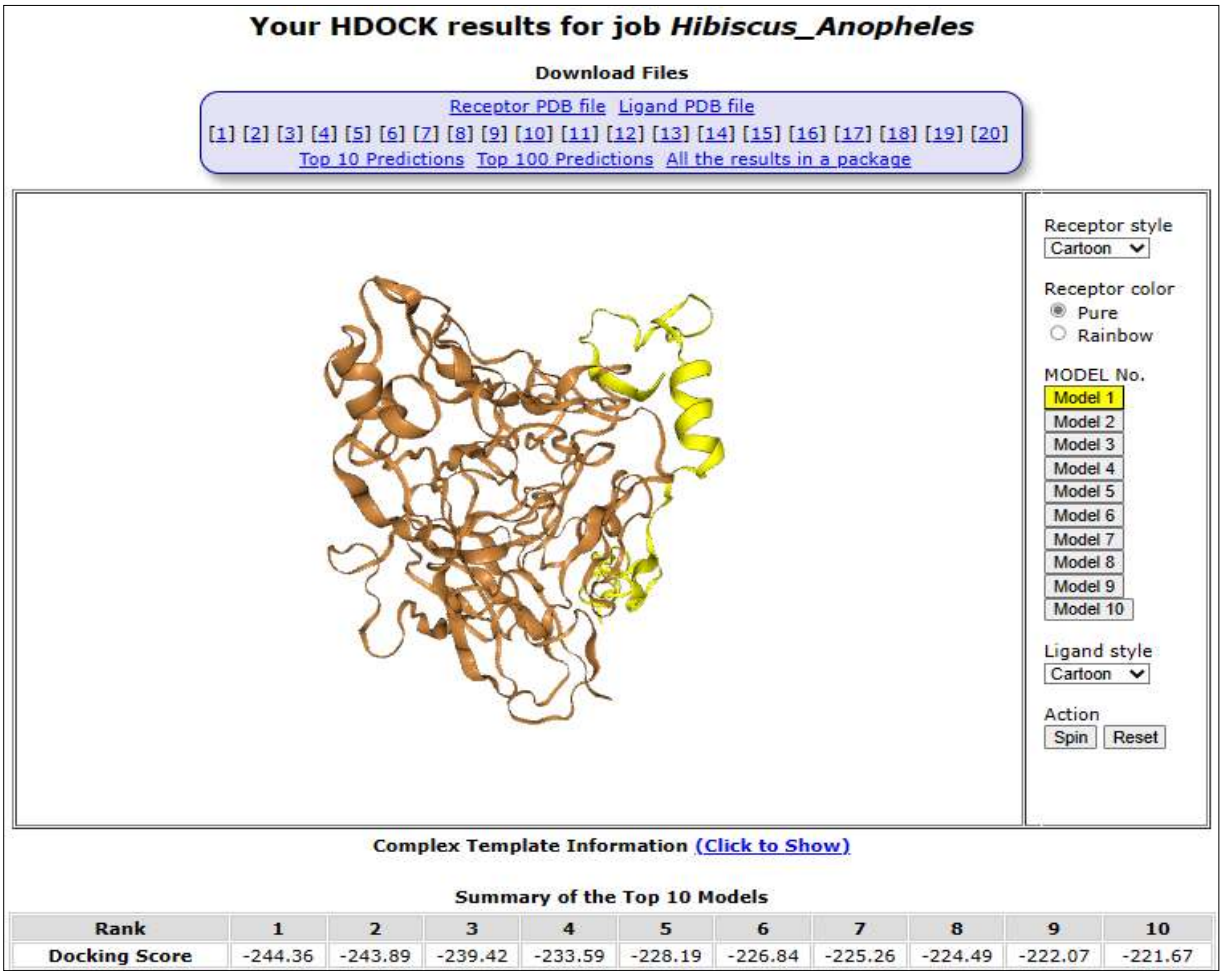
## Figures

>ACU33027.1 sulfite oxidase [Hibiscus cannabinus]
MPGIKGPSDYSQEPPRHPCLQINSKEPFNAEPPRSALVSSYVTRVDLFYKRNHGPIPVVDDIERYCFDIS
GLIQTPKKLYMRDVRMLPKYNVTAILQCAGNRRTAMSKTRKVRGVGWDVSAIGNAVWGGAKLADVLELVG
IPKLTSRTQSGGKHVEFVSIDKCKEENGGPYKASIPLIQATNPEADVLLAYEMNGEPLNRDHGYPLRVIV
PGVIGARSVKWLDSINILAEECQGSFMQKDYKMFPPSVDWNINWSTRRPQMDFPVQSVICSLEDVQSIK
PGKITIGYAASGGGRGIERVDVSIDGGKTWLEASRSQKTGIPYISDHESSDKWAWVLFEITVDIPHSTE
IVAKAVDSAANVQPENVQDIWNLRGILNTSWRVQIRVGHSNM

**Fig 1:** Amino acid sequence of Sulfite oxidase of *Hibiscus cannabinus.*

>XP_049540068.1 pro-resilin [Anopheles darlingi]
MMKFVVLAVCLCVVVIVDQTLAQNNQYLPPDKGYAYDKPNQPFPSSPQPQPRPPQPTPGRPAPSYGPPPA
TDDHHHEPGMPFDFQYNVNDIETQNDYSHKAVSDGDVTRGEYRVQLPDGRTQIVRYTADWKNGYNAEVSY
EGEAKYPEGPGQGGANAGGYKY

**Fig 2:** Amino acid sequence of Pro-resilin of *Anopheles darlingi*




| Rank | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Docking Score | -244.36 | -243.89 | -239.42 | -233.59 | -228.19 | -226.84 | -225.26 | -224.49 | -222.07 | -221.67 |

**Fig 3:** 3D Molecular docking interactions between Sulfite oxidase and Pro-resilin using HDOCK server

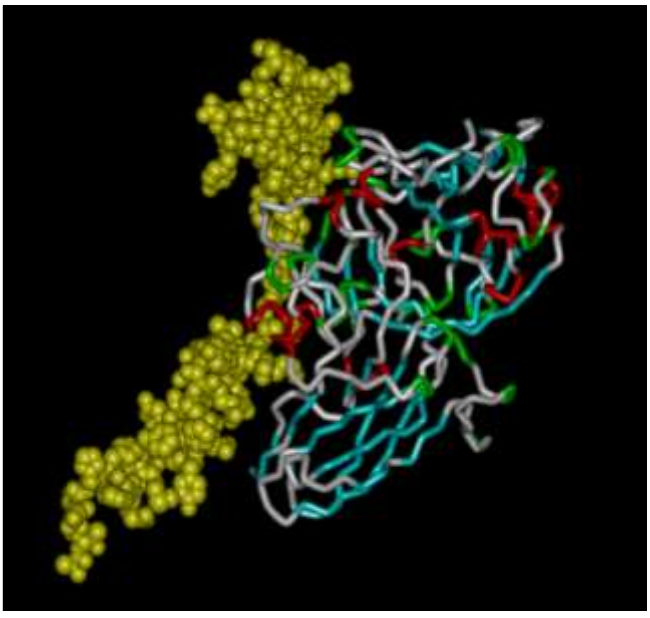


**Fig 4:** 3D Molecular docking interactions between Sulfite oxidase and Pro-resilin using Discovery Studio software

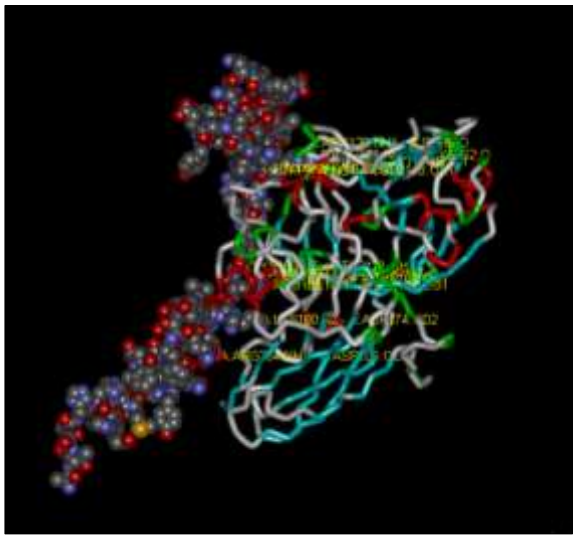


**Fig 5:** 3D Molecular docking interactions between Sulfite oxidase and Pro-resilin using Discovery studio software (Amino acids interacting at H-bond : LYS: 108, TRP:254, ARG:384, LYS:100, ARG:109, ARG:120, GLU:148, TYP:112, GLU:148, ASP:106.ASP:274, THR:104, TRP:117, SER:8, LYS:162, ILE:252)

## Conclusion

In the Amazon area, *Anopheles darlingi* is a significant malaria vector. The pro-resilin of *Anopheles darlingi* directly attaches to the chosen chemical molecule, Sulfite oxidase, as demonstrated by this scientific inquiry. All eukaryotes have sulfite oxidase, an enzyme, in their mitochondria. The goal of the current study was to use naturally occurring substances, such as the non-toxic Sulfite oxidase, to suppress mosquito populations. The body of research unequivocally demonstrates that Sulfite oxidase directly binds to the functional portion of *Anopheles darlingi* pro-resilin, down regulating it. Hence, Sulfite oxidase may be employed as an effective mosquito-controlling agent.

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