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Assessment of bio-larvicide for *Culex epidesmus* through bioassay along with toxicokinetics and virtual screening of phytoligands from the leaf of *Azadirachta indica* against mosquito acetylcholinesterase

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***Abstract*:** The bio-larvicide is a substitute of synthetic larvicide to prevent growth of mosquito larvae. The present study was aimed to detect percentage mortality of larvae of *Culex epidesmus* Theobald, 1910 by aqueous extract of neem leaf (*Azadirachta indica* A. Juss) and the inhibitory potential of established the phytoconstituents present in the leaf of neem against the protein of mosquito (acetylcholinesterase) through molecular docking and toxicokinetics. The acetylcholinesterase (receptor) was obtained (PDB ID: 2AZG) from the Protein Data Bank (PDB) and the information of ligands (phytochemicals) were obtained from PubChem database. Few established phytochemicals of leaf were used as ligands in this study. These chemical structures (three-dimensional) were procured from online CORINA software. The software, PyRx (Version 0.8) for the structure-based virtual screening and ADMET-SAR were used for toxicokinetics study. The present results indicate that phytochemicals found in the leaf extract of *A. indica* observed mortality to the larvae at higher concentrations (70–100%). The interaction with different residues of mosquito acetylcholinesterase protein observed ligand binding energy for quercetin (-9.4 Kcal/mol). Among other phytochemicals, quercetin may have an inhibitory effect on nerve protein for larvae mortality and may be suitable compound for environment by toxicokinetics evaluation. In conclusion, it was obtained through faster screening by using software that phytoconstitent quercetin of *A. indica* may use future larvicide as a lead compound for the prevention of *C. epidesmus* growth. It is suggested that functional assay (*in vivo* and *in vitro* assay) should be carried out for the validation of the present results.

Keywords: neem leaf extract; bioassay; mosquito bio-larvicide; acetylcholinesterase protein; virtual screening; toxicokinetics; phytochemicals

I. INTRODUCTION

Several species of mosquito are the causative agents for many diseases like dengue, malaria fever, filariasis, etc. It was known that the Culex mosquito-transmitted several diseases worldwide also in many parts of India. Sometimes the larvae are threatening the human life. There are several synthetic larvicides in use to prevent the extra burden of larvae. These diseases occur sometimes epidemiological impact in the globe. Among these species, *Culex epidesmus* is one of the vector that transmit disease to human.

Till date, different chemical and biological agents have been discovered from synthetic or natural origin to destroy the larvae of different mosquito species [1-2]. The chemicals are well-known larvicides such as organochlorine, organophosphate, carbamate and pyrethroids [1; 3-4], but these synthetic chemicals show toxicity to other aquatic species along with food chains and also develop genes for a species-resistant [3-4; 5-6]. On the other hand, several larvicides from plant extracts have potent larvicidal effect to the larvae without hampering others biota and unable to develop resistance like synthetic insecticides [7-9].

In the development of bio-larvicides, among other plant species, different parts of neem plant (*A. indica*) have already been well-established in the field of mosquito-borne disease prevention without damaging the environment [8-9; 10-14]. Generally, the growth inhibition of larvae by using insecticides, a mechanism to inhibit the acetylcholinesterase enzyme (AChE), a neurotransmitter found in insects, fish, birds, mammals. In other word, AChE is an insect-specific cysteine residue, which opens at acetylcholinesterase active site. This is basically a promising target site for creating new insecticides with reduced off-target toxic response and low propensity for insect resistance [15-21].

There have several reports emphasized that *A. indica* leaf contained various phytochemicals, which prevents different diseases [22-25]. Few researches are investigating to develop larvicide from neem leaf extract through toxicity study [8-9] and work is lacking to detect exact phytocompound is having a potent larvicidal activity to inhibit AChE of mosquito protein through molecular docking and toxicokinetics study by using software.

The present study was aimed to detect percentage mortality of larvae of *Culex epidesmus* (Theobald, 1910) by aqueous extract of neem leaf and the inhibitory potential and toxicity of established the phytoconstituent present in the leaf of *Azadirachta indica* A. Juss. against the protein of mosquito (acetylcholinesterase) through molecular docking and toxicokinetics.

II. MATERIALS AND METHODS

Neem leaf sample collection and preparation of extract

The neem leaf sample was collected from the college campus, Serampore, West Bengal, India. The aqueous neem (*A. indica*) leaf extract was prepared by using fresh leaves. The extraction was done by the method of Rashid and Ahmad, [8] with some modifications. All the leaves were cleaned by keeping with the running tap water, followed by distilled water, then kept on the blotting paper to soak the excess water. The leaves of 20 nos. were kept in mortar and macerated by pastel along with dechlorinated tap water. The

solution was filtered and taken in a clean glass bottle as a stock solution (100%). The organic solvents were not used.

Toxicity test for larvae of *Culex epidesmus*

From this stock solution, different dilutions were prepared as 70%, 40%, 20% and 10%. The supplied larvae (*Culex epidesmus*) were kept in the aerated water prior to toxicity test and 10 nos. were used in each petri dish as per higher to lower dilutions (100% - 10%). The test was performed twice as replicate. The percentage mortality was recorded in each dilution for 0hr, 24hr and 48hr.

Protein selection

The crystal structure of mosquito protein acetylcholinesterase (PDB ID: 2AZG) was selected (Figure 1) and retrieved from protein data bank (http://www.rcsb.org/) because this protein shows response in the larvae of mosquito due to inhibition by chemical(s). The crystal structure has been refined, finally the model deposited in the form of PDB on 10th September 2005 (PDB ID: 2AZG) and released on 19th September 2006 as PDB [26].

Phytoligands selection

Established 12 phytocompounds from *A. indica* were selected from the literature reported by Subapriya and Nagini [23]. The three-dimensional (3-D) structure of twelve ligands were retrieved from the NCBI PubChem database (http://www.ncbi.nlm.nih.gov/ pccompound/), all the ligand molecules were converted into 3-D structure using the 3-D converter module, CORINA online software (http://www.mol-net.de) after incorporating the Canonical SMILES string for each chemical that taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/compound) and the structures of the ligands are depicted in Figure 2.

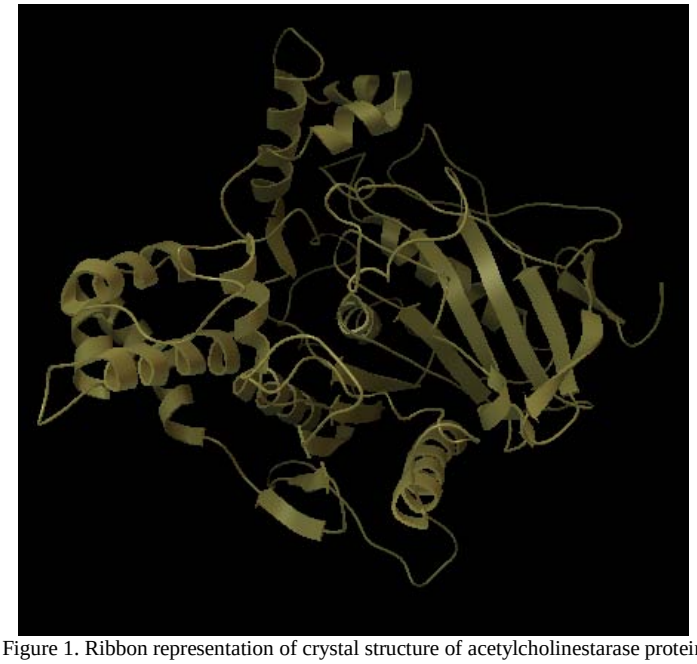
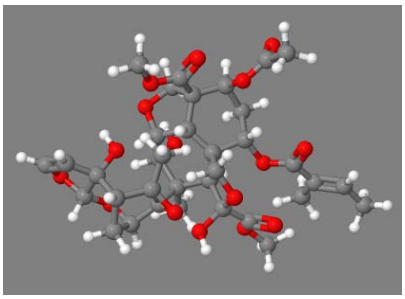
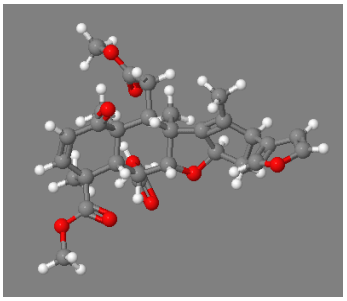


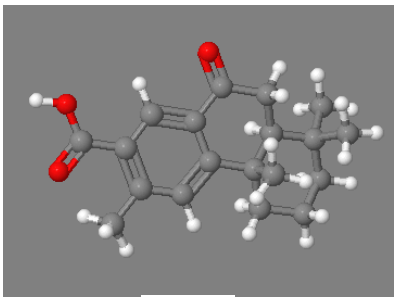
Figure 1. Ribbon representation of crystal structure of acetylcholinestarase protein



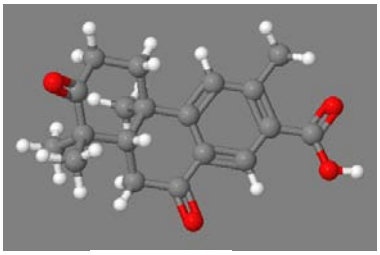
Azadirachtin A



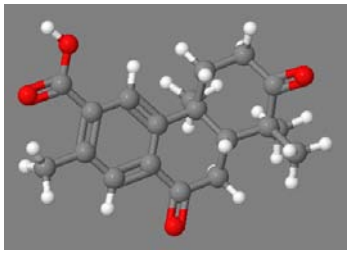
Nimbin



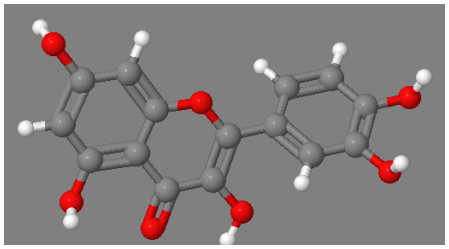
Margolone



Margolonone



Isomargolonone



Quercetin

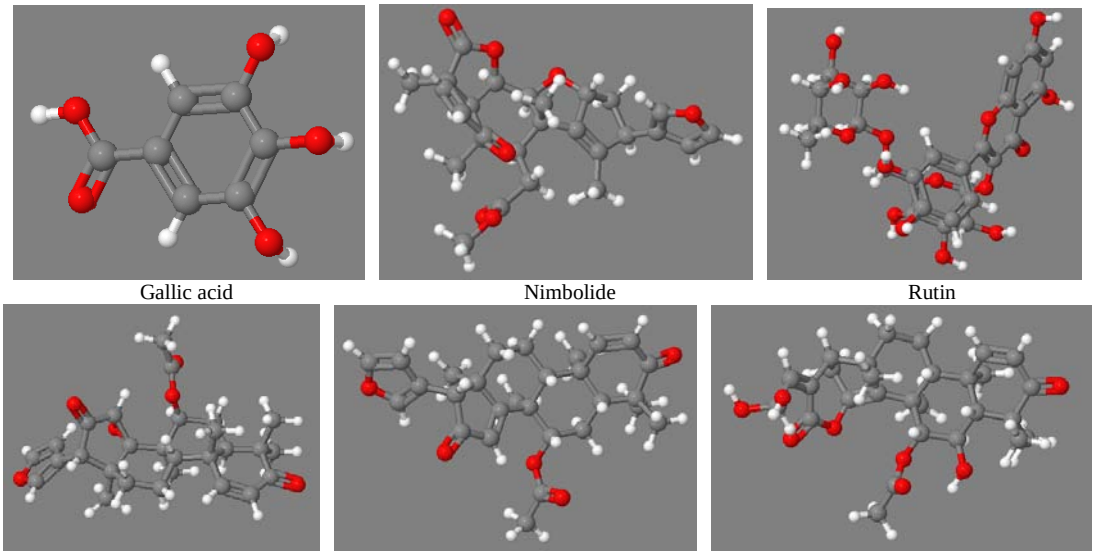


Figure 2. Structural representation of phytocompounds found in the leaf of *A. indica*

Molecular docking

The molecular docking was done through PyRx software (Version 0.8) developed by Trott and Olson [27]. The molecular docking result for each compound was visualized through pdbqt output by using this tool. The docking site on this target was expressed by forming a grid box with the dimensions of X: 63.3502 Y: 74.6010 Z: 72.2942 Å, with a grid spacing of 0.375 Å, centered on X: 116.14 Y: 103.95 Z: -142.83 Å. The PyRx software is a docking program that basically predicts receptor-ligand binding along with providing energy value for each test compound.

Toxicokinetics study

ADMET-SAR (absorption, distribution, metabolism, excretion, toxicity – structure activity relationship) to check whether the compound has fulfilled the conditions as a candidate for biopesticide or larvicide [28].

III. RESULTS AND DISCUSSION

Toxicity test for larvae of *Culex epidesmus*

The 24hr recorded percentage mortality i.e. 20%, 40%, 50%, 70% and 100% in extract having 10%, 20%, 40%, 70% and 100% dilution while the 48hr recorded percentage mortality i.e. 40%, 60%, 80%, 100% and 100% in extract having percentage of 10, 20, 40, 70 and 100 dilutions (Table I). The present acute toxicity results indicated highest percentage mortality (100%) of the larvae of *Culex epidesmus* within 24hr duration 100% extract was obtained 100% mortality while 48hr duration 70% - 100% dilution of leaf extract of *A. indica* showed 100% mortality. The neem leaf extracts of different dilutions caused mortality to the larvae of different species of mosquito, which is supporting the present results of larvicidal activity [8-14]. The R^2 values were observed 97% and 85% for 24hr and 48h duration respectively. The dose-response curve is depicted in Figure. 3. Synthetic chemicals are used to eradicate the larval population of mosquito to prevent human from mosquito-borne diseases [1]. In general, several synthetic pyrethroids are well-known toxins to enhance mortality of larvae in their aquatic habitat, but these chemicals may also hamper other aquatic life. The phytochemicals of *A. indica* leaf have the potent ability to destroy mosquito larvae of *Culex epidesmus* as biological agents or can be used as larvicide for mosquito control.

Table I. Percentage dilutions of neem leaf extract versus percentage mortality of larvae

Extract concentrations (% dilution)	**Species used (in nos.)**	**Time of exposure (in hr)**		
		0	**24**	**48**
		% mortality	**% mortality**	**% mortality**
Control (0)	10	0	0	0
10	10	0	20	40
20	10	0	40	60
40	10	0	50	80
70	10	0	70	100
100	10	0	100	100

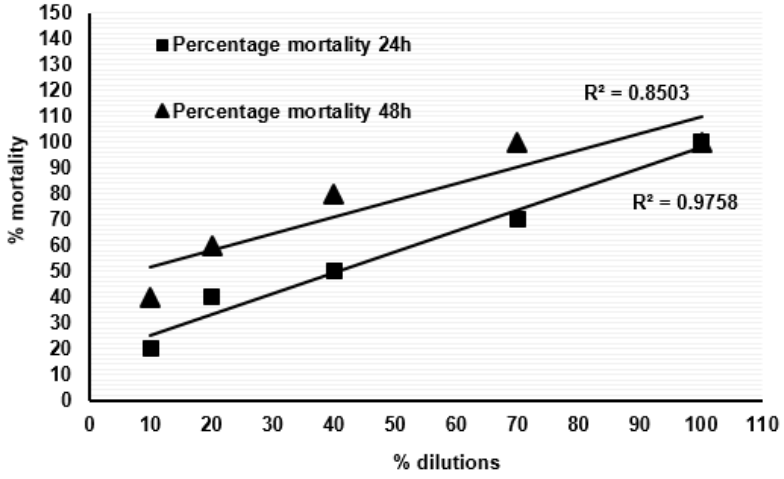


Figure 3. Dose-response curve for larvae of *C. epidesmus*

Virtual screening

It was observed from Table II that the high binding energy value (kcal/mol) was obtained in quercetin (-9.4) followed by margolone (-9.0), rutin (-8.7), Azadiradione (-8.6), margolonone and nimbolide (-8.2), isomargolonone (-8.1), nimbinin (-7.9), azadirachtinA (-6.9) and gallic acid (-6.1). 3-D ribbon structure for binding position for all above phytoligands was obtained through PyRx software and the 3-D structure of binding interaction procured. The ligand quercetin showed two numbers of hydrogen bonding between hydrogen atom of Tyr130 and Tyr328 (Figure 4 A and B). Among other phytoligands, only gallic acid was observed similarity with quercetin having bonding between a hydrogen atom of Tyr130. This prediction results along with the toxicity of aqueous extract revealed that quercetin is more suitable phytoligand present in *A. indica* that may have inhibitory activity of acetylcholinesterase protein found in insects. The researchers have studied reversible and irreversible inhibition in the acetylcholinesterase protein [18-21; 26] and there have been previously found compounds that developed resistance in the genes of AChE. The isolation of this particular phytochemical can be developed from neem leaves for mosquito larvae especially *C. epidesmus* control as bio-larvicide.

These parameters are well-known high throughput predictions of absorption, metabolism, distribution, excretion and toxicity that helps in the detection of active lead compounds at early chemical formulation. According to Tsaioun et al. [30] (2009), previously ADME study benefitted in relation to the development of effective lead compounds in drug discovery. In this context, researchers have stated that ADMET properties prediction with special reference to Blood-Brain barrier (BBB) penetration, P-glycoprotein substrate, renal organic cation transporter, human intestinal absorption and Caco2 permeability of docked compounds are suitable pharmacological parameters for drug designing [29; 31-33]. Herein, it is an endeavour to develop larvicide for mosquito larvae eradication from water without hampering the other biota in waterbodies. In case of metabolism, other important parameters are cytochrome P450 (CYP) of different types, which belongs to isozymes group and it participates in the metabolism of drugs, fatty acids, steroids, bile acids and carcinogens [33].

Table II. Molecular docking for leaf phytochemicals of *A. indica* against acetylcholinesterase protein of mosquito

Sl. No.	Ligands	Binding affinity (Kcal/mol)	H-bond residues
1.	Quercetin	-9.4	Tyr130 & Tyr328
2.	Margolone	-9.0	Arg 339
3.	Rutin	-8.7	Asp71 & Val72
4.	Azadiradione	-8.6	His132
5.	Margolonone	-8.2	Asn98
6.	Nimbolide	-8.2	---
7.	Isomargolonone	-8.1	Asn98
8.	Nimbinin	-7.9	---
9.	Nimocinolide	-7.7	Leu234
10.	Nimbin	-7.2	Asn87 & Arg133
11.	AzadirachtinA	-6.9	---
12.	Gallic acid	-6.1	Tyr130

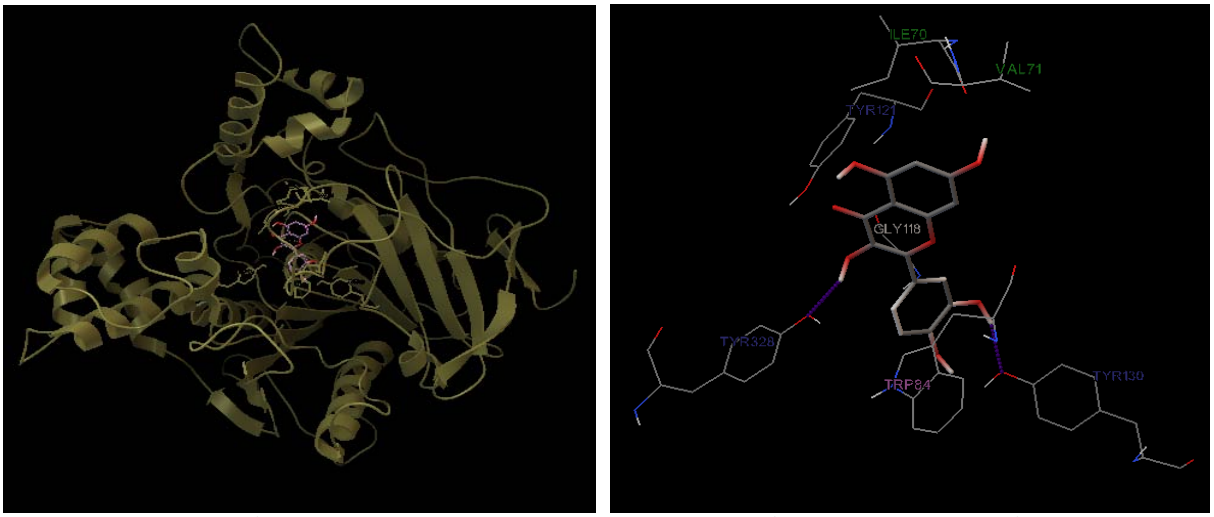


Figure 4. Pictorial representation of receptor-ligand binding interaction. A = Ribbon structure of protein and line structure of ligand; B = Hydrogen bond interactions between quercetin and the active site residues of acetylcholinesterase. Blue dotted lines represent the hydrogen bonds interactions between hydrogen atom of Tyr130 and Tyr328

IV. CONCLUSION

It is concluded that quercetin compound was observed a suitable for inhibitory effect on acetylcholinesterase protein (PDB ID: 2AZG) through in silico study with special reference to molecular docking and toxicokinetic evaluation. It is well-established that the crude aqueous extract of neem leaf is having larvicidal activity for the larvae of mosquito [8-14]. However, earlier several synthetic larvicides have been used to control mosquito larvae, but these have developed compound resistance to AChE genes [3]. To prevent gene resistant by the compound, the development of bio-larvicide can be a good effort. On the other hand, these phytochemicals do not have much toxicity, mutagenicity and/or carcinogenicity to the mammals especially human. It is suggested *in vitro* and *in vivo* study, excluding to remove resistance genes of AChE prior to developing larvicide.

Table III. ADMET-Prediction profiles for phytochemicals of *A. indica* leaf

Absorption								**Distribution**
Sl. No.	**Phytoligands**	**Blood-brain barrier**	**Caco-2 permeability**	**Human intestinal absorption**	**P-glycoprotein inhibitor**		**P-glycoprotein substrate**	**Subcellular localization**
1.	Quercetin	BBB-	Caco2-	HIA+	NI		S & NI	Mitochondria
2.	Margolone	BBB+	Caco2+	HIA+	NI		S & NI	Mitochondria
3.	Rutin	BBB-	Caco2-	HIA+	NI		S & NI	Mitochondria
4.	Azadiradione	BBB+	Caco2+	HIA+	I		SI	Mitochondria
5.	Margolonone	BBB+	Caco2+	HIA+	NI		S & NI	Mitochondria
6.	Nimbolide	BBB+	Caco2+	HIA+	I		SI	Mitochondria
7.	Isomargolonone	BBB+	Caco2+	HIA+	NI		S & NI	Mitochondria
8.	Nimbinin	BBB+	Caco2-	HIA+	I		SI	Mitochondria
9.	Nimocinolide	BBB+	Caco2-	HIA+	I		SI	Mitochondria
10.	Nimbin	BBB+	Caco2+	HIA+	I		SI	Mitochondria
11.	AzadirachtinA	BBB-	Caco2-	HIA+	NI		SI	Mitochondria
12.	Gallic acid	BBB-	Caco2-	HIA+	NI		NS & NI	Mitochondria
Metabolism								**Excretion**
Sl. No.	**Phytoligands**	**CYP450 2C9 inhibitor**	**CYP450 2C9 substrate**	**CYP450 2D6 inhibitor**	**CYP450 2D6 substrate**	**CYP450 3A4 inhibitor**	**CYP450 3A4 substrate**	**ROCT**
1.	Quercetin	NI	NS	NI	NS	I	NS	NI
2.	Margolone	NI	NS	NI	NS	NI	S	NI
3.	Rutin	NI	NS	NI	NS	NI	NS	NI
4.	Azadiradione	NI	NS	NI	NS	I	S	NI
5.	Margolonone	NI	NS	NI	NS	NI	S	NI
6.	Nimbolide	NI	NS	NI	NS	I	S	NI
7.	Isomargolonone	NI	NS	NI	NS	NI	S	NI
8.	Nimbinin	NI	NS	NI	NS	I	S	NI
9.	Nimocinolide	NI	NS	NI	NS	NI	S	NI
10.	Nimbin	NI	NS	NI	NS	I	S	NI
11.	AzadirachtinA	NI	NS	NI	NS	NI	S	NI
12.	Gallic acid	NI	NS	NI	NS	NI	NS	NI

Toxicity						
Sl. No.	**Phytoligands**	**Acute oral toxicity**	**Fish toxicity**	**Honey bee toxicity**	**AMES toxicity**	**Carcinogens**
1.	Quercetin	II	HFMHT	HHBT	NT	NC
2.	Margolone	III	HFMHT	HHBT	NT	NC
3.	Rutin	III	HFMHT	HHBT	NT	NC
4.	Azadiradione	III	HFMHT	HHBT	NT	NC
5.	Margolonone	III	HFMHT	HHBT	NT	NC
6.	Nimbolide	III	HFMHT	HHBT	NT	NC
7.	Isomargolonone	III	HFMHT	HHBT	NT	NC
8.	Nimbinin	III	HFMHT	HHBT	NT	NC
9.	Nimocinolide	III	HFMHT	HHBT	NT	NC
10.	Nimbin	III	HFMHT	HHBT	NT	NC
11.	AzadirachtinA	I	HFMHT	HHBT	NT	NC
12.	Gallic acid	III	HFMHT	HHBT	NT	NC

NI = Non-inhibitor; I = Inhibitor; NS = Non-substrate; S = Substrate; SI = Substrate inhibitor; ROCT = Renal Organic Cation Transporter; I = Category I (LD_{50} values less than or equal to 50mg/kg); II = Category II (LD_{50} values greater than 50mg/kg but less than 500mg/kg); III = Category III (LD_{50} values greater than 500mg/kg but less than 5000mg/kg) and IV = Category IV (LD_{50} values greater than 5000mg/kg); H = High; L = Low; FHMT = Fathead minnow toxicity; HBT = Honey bee toxicity; NT = Non-toxic; NC = Non-carcinogen

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest for the present study.

VI. REFERENCES

1. J. Hemingway and G. P. Georghiou, "Studies on the acetylcholinesterase of *Anopheles albimanus* resistant and susceptible to organophosphate and carbamate insecticides", Pesticide Biochemistry and Physiology, 19 (2), 167-171, 1983.
2. A. Ghosh, N. Chowdhury and G. Chandra, "Plant extracts as potential mosquito larvicides," Indian Journal of Medical Research, 135 (5), 581-598, 2012.
3. H. Ranson, B. Jensen, J. M. Vulule, X. Wang, J. Hemingway and F. H. Collins, "Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids," Insect Molecular Biology, 9 (5), 491-497, 2000.
4. J. Intirach, A. Junkum, B. Tuetun, W. Choochote, U. Chaithong, A. Jitpakdi, D. Riyong, D. Champakaew and B. Pitasawat, "Chemical constituents and combined larvicidal effects of selected essential oils against *Anopheles cracens* (Diptera: Culicidae)," Psyche, 2012, Article ID 591616, 11 pages, 2012. doi:10.1155/2012/591616
5. H. R. Rathore, G. Toqir, S. Rashid, S. M. Mujtaba and S. M. Nasir, "Insecticide resistance in *Anopheles* mosquito of Punjab province," Pakistan. Pakistan Journal of Zoology, 17 (1), 35-50, 1986.
6. M. Weill, G. Lutfalla, K. Mogensen, F. Chandre, A. Berthomieu, C. Berticat, N. Pasteur, A., Philips and M. Raymond, "Comparative genomics: Insecticide resistance in mosquito vectors," Nature, 423, 136-137, 2003.
7. J. A. N. H. Oudegans, "Agro-pesticides". United Nation Economic and Social Commission for Asia and the Pacific. Bangkok, 31pp, 1991.
8. M. Rashid and A. Ahmad, "The effect of neem (*Azadirachta indica*) leaves extract on the ecdysis and mortality of immature stages of common house mosquito *Culex pipiens fatigans*," Biologia (Pakistan) 59 (2), 213-219, 2013.
9. M. E. Medani, M. E. Elkhidir, A. B. Satti, "Sensitivity of mosquito larvae to water extract of *Azadiracta indica* (Neem)," Journal of Science, 5 (3), 147-150, 2015.
10. S. R. Chavan, "Chemistry of alkanes separated from leaves of *Azadirachta indica* and their larvicidal/insecticidal activity against mosquitoes," Schriftenr GTZ, 161, 59-65, 1984.
11. B. L. Aliero, "Larvicidal effects of aqueous extracts of *Azadirachta indica* (neem) on the Larvae of *Anopheles* mosquito," AFRJ Biotechnol., 2 (9), 325-327, 2003.
12. H. Vatandoost and V. M. Vaziri, "Larvicidal activity of a neem tree extract (Neemarin) against mosquito larvae in the Islamic Republic of Iran," Eastern Mediterranean Health Journal, 10 (4/5), 573-581, 2004.
13. T. X. Lin and S. S. Liu, "Properties and potential of natural pesticides from neem tree, *Azadirachta indica*," Pest Management Science, 62(1), 38pp, 2006.
14. A. Abdelouaheb, R. Nassima and S. Noureddine, "Larvicidal activity of a neem tree extract (Azadirachtin) against mosquito larvae in the Republic of Algeria," Jordan Journal of Biological Sciences, 2(1), 15-22, 2009.
15. J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker and I. Silman "Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein," Science, 253, 872-879, 1991.
16. P. Taylor and Z. Radic, "The cholinesterases: from genes to proteins," Annual Review of Pharmacology, 34, 281-320, 1994.

17. M. L. Raves, M. Harel, Y.-P. Pang, I. Silman, A. P. Kozikowski and J. L. Sussman, "Structure of acetylcholinesterase complexed with the nootropic alkaloid, (-)-huperzine A," Nature Structural Biology, 4, 57-63, 1997.
18. Y.-P. Pang, F. Ekstrom, G. A. Polsinelli, Y. Gao, S. Rana, D. H. Hua, B. Andersson, P. O. Andersson, L. Peng, S. K. Singh, R. K. Mishra, K. Y. Zhu, A. M. Fallon, D. W. Ragsdale and S. Brimijoin "Selective and irreversible inhibitors of mosquito acetylcholinesterases for controlling malaria and other mosquito-borne diseases," PLoSOne 4 (8), e6851, 2009. (doi:org/10.1371/journal.pone.0006851).
19. Y.-P. Pang, S. Brimijoin, D. W. Ragsdale, K. Y. Zhu and R. Suranyi, "Novel and viable acetylcholinesterase target site for developing effective and environmentally safe insecticides," Current Drug Targets, 13, 471-482, 2012.
20. M. B. Colovic, D. Z. Krstic, T. D. Lazarevic-Pasti1, A. M. Bondzic and V. M. Vasic, "Acetylcholinesterase inhibitors: Pharmacology and toxicology," Current Neuropharmacology, 11, 315-335, 2013.
21. D. Dou, J. G. Park, S. Rana, B. J. Maden, H. Jiang and Y.-P. Pang, "Novel selective and irreversible mosquito acetylcholinesterase inhibitors for controlling malaria and other mosquito-borne diseases," Scientific Reports, 3, 1068, 2013 (doi:10.1038/srep01068).
22. K. Biswas, I. Chattopadhyay, R. K. Banerjee and U. Bandyopadhyay, "Biological activities and medicinal properties of neem (*Azadirachta indica*)," Current Science, 82, 1336-1345, 2002.
23. R. Subapriya, and S. Nagini, "Medicinal properties of neem leaves: A review," Current Medicinal Chemistry. Anti-cancer Agents, 5, 149-156, 2005.
24. D. A. Mahmoud, N. M. Hassanein, K. A. Youssef and A. Zeid, "Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens," Brazilian Journal of Microbiology, 42, 1007-1016, 2011.
25. P. S. Bhajoni, G. G. Meshram and M. Lahkar, "Evaluation of the antiulcer activity of the leaves of *Azadirachta indica:* An experimental study," Integrative Medicine International, 3, 10-16, 2016.
26. Y-P. Pang, "Novel acetylcholinesterase target site for malaria mosquito control," PLoS One, 2006 (1), e58, 2006. (doi:10.1371/journal.pone.0000058).
27. O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading," Journal of Computational Chemistry, 31, 455-461, 2010.
28. F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P. W. Lee and Y. Tangadmet, "SAR: a comprehensive source and free tool for assessment of chemical ADMET properties," Journal of Chemical Information and Modeling, 52 (11), 3099-3105, 2012.
29. P. Anitha, P. Lavanya, A. Anbarasu and S. Ramaiah, "Molecular docking study of catechins compounds from *Camellia sinensis* against UPPS in *Staphylococcus aureus*," International Journal for Computational Biology, 3 (2), 03-09, 2014.
30. K. Tsaioun, M. Bottlaender and A. Mabondzo, "Alzheimer's Drug Discovery Foundation. ADDME--Avoiding Drug Development Mistakes Early: central nervous system drug discovery perspective," BMC Neurology, 9 (1), S1, 2009.
31. M. S. Alavijeh, M. Chishty, M. Z. Qaiser and A. M. Palmer, "Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery," NeuroRx, 2 (4), 554-557, 2005.
32. S. Hurst, C. M. Loi, J. Brodfuehrer and A. El-Kattan, "Impact of physiological, physicochemical and biopharmaceutical factors in absorption and metabolism mechanisms on the drug oral bioavailability of rats and humans," Expert Opinion on Drug Metabolism and Toxicology, 3 (4), 469-489, 2007.
33. F. P. Guengerich, "Cytochromes P450, drugs, and diseases," Molecular Intervention, 3, 194-204, 2003.