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Isolation of Bioactive Constituent from the Leaves of *Acalypha indica* L. and Evaluated *in vitro* Larvicidal and Molecular Docking Studies

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The process of mosquito control using synthetic substances having lot of hazardous effects to human and the environment. So, there is an urgent need to develop plant based insecticides. The ethanolic extract of medicinal plant *Acalypha indica* leaves was imperiled to preliminary phytochemical screening, bioactive fraction isolation, larvicidal activity and molecular docking studies. Preliminary phytochemical screening displayed the existence of steroids, flavonoids, alkaloids, sugars and saponins in ethanolic extract of *Acalypha indica* L. The isolated bioactive compound was confirmed by using spectroscopic methods such as GC-MS, FT-IR, NMR (^{1}H & ^{13}C) and elemental analyses. The spectroscopic data confirmed the isolated compound was *bis*(2-ethylhexyl)phthalate (**1**). Compound **1** exerted more larvicidal activity than plant powder and its ethanolic extract with an LD_{50} value of 38.23 µg/mL, which was better than that of control permethrin with an LD_{50} value of 54.05 µg/mL. Plant powder and ethanolic extract were moderate larvicidal effect towards *C. quinquefasciatus* with LD_{50} values of >100 and 75.13 µg/mL, respectively. The molecular docking studies shows significant supporting evidence for larvicidal activity.

Keywords: *Acalypha indica* L., *Culex quinquefasciatus*, Larvicidal activity, Odorant binding protein, Molecular docking.

INTRODUCTION

Medicinal herbs and other natural remedies were rejected by males when the first signs of social conflict emerged. Each civilization, even the western one, has developed its own unique system of conventional therapeutic treatment [1]. There is a long history of people from many civilizations making basic medicine as a result of its struggle alongside natural disasters and diseases, which is at the heart of traditional treatment. Also, the ancient period learned that many foods might treat or prevent illness. This led to the widespread adoption of diets rich in medicinal herbs and spices [2]. There has been a lot of studies on the potential therapeutic effects of secondary metabolites from plants. For the treatment of fungal, bacterial and viral illnesses, it is expected that phytonutrients with adequate antibacterial effect will be discarded [3]. In recent decades, the usage of herbal remedies has increased dramatically around the globe, especially in progressive countries. Pledge-categorized crops also saw a significant increase in the global commerce and lucrative exploitation of natural remedies. Other nations, including Papua New Guinea, Argentina, Ethiopia and China, still use herbal remedies as an integral part of their medical system [4-7]. When one or more of a plant's components include substances that may be used as charities for therapeutic aspirations and progenitors for the development of useful medications, the World Health Organization (WHO) defines such herb as a therapeutic herb. These days, herbal plagiaristic medicines are the primary form of health insurance for over 90% of the world's population. People in Asia and India are increasingly turning to plant-based diets as a means of breaking the monotony of their health maintenance routines [8,9].

Acalypha indica is a wildflower herb that ensues all over the flat of China, South Africa and India. It have its place to the Euphorbiaceae family. *Acalypha indica* widely castoff habitually on behalf of handling several illnesses for instance bacterial infections, wound healing, cancer, post-coita, antivenom, diuretic effects, infertility, inflammation and antioxidant [10-16]. Introductory phytochemical analysis of *Acalypha*

indica leaves displays the existence of flavonoids, saponins, terpenoids tannins, polyphenols and alkaloids [17].

Mosquitoes are regarded as one of the most significant orders of insects by medical and animal entomologists. Worldwide, mosquitoes are the biggest problem because of the many terrible diseases they spread to humans and animals [18]. They are responsible for the transmission of encephalitis-causing pathogens such West Nile virus, filariasis, malaria, yellow fever, chikungunya, filariasis and dengue fever [19]. Over two million individuals and one million kids die each year from mosquito-borne illnesses [20]. These illnesses are prevalent in more than 100 nations. Mosquitoes of the genera Aedes, Anopheles and Culex are the most common insect vectors of human illness. As a result, they devour a significant risk to worldwide public health and primarily affect the economies and communities of tropical and subtropical regions. Because of these issues, researchers have been looking for and developing new ways to combat mosquito larvae through ecological, non-toxic, biodegradable and inexpensive solutions [21].

Many biological functions, including as feeding, mating, reproducing and detecting danger, rely on olfactory data [22]. The odorants are transported to the olfactory receptors by odorant-binding proteins (OBP) [23,24]. As a result, it is decided to conduct molecular docking experiments on the OBP of *C. quinquefasciatus* (PDB ID: 3OGN). So the present work was designed to determine the preliminary phytochemical screening and isolation of bioactive compound from ethanolic leaf extract of medicinal plant *A. indica* and evaluated for its larvicidal and molecular docking studies.

EXPERIMENTAL

All chemicals and solvents were procured from Sigma-Aldrich and utilized as received. FT-IR spectra were captured using a Shimadzu 8201pc spectrometer (4000-400 cm^{-1}). In this case, a JEOL-400/100 MHz spectrometer was used to record the NMR (^{1}H and ^{13}C) spectra. An elemental analyzer (Vario EL III) was employed to identify the elements carbon, hydrogen and nitrogen. Thin-layer chromatography (TLC) using silica gel plates was used to verify the purity of the chemicals.

Collection of plant material: The plants employed in this research were from the area around Trichy. The Rapinart Herbarium at St. Joseph College in Trichy was used to verify the plant's identities. Fresh *Acalypha indica L.* leaves were gathered, cleaned and dried in the shade. The powdered dry leaves were utilized in subsequent research.

Preparation of extracts: The powdered components were extracted using ethanol at 70-80 ºC using a hot continuous process in a Soxhlet extractor for 24 h. The extract was frozen in a lyophilizer and then evaporated to a concentrated powder using a rotary evaporator. Finally, chromatographic analyses were performed on the remaining residue.

Phytochemical analysis: Preliminary phytochemical analysis of enthanolic extract was carried out as per the standard texual procedure [25-28].

Isolation of bioactive compound: To achieve the homogeneous mixing, 5 g of ethanolic extract of *Acalypha indica* L. leaves were collected individually and blended with 5 g silica gel (60/120 meshes). Hexane was used as the packing solvent and 200 g of silica gel (70/325 meshes) were properly packed without air bubbles in an appropriate column [29,30]. We set aside the column for 1 h to ensure snug packing. The addition of admixture on top of the stationary phase initiated the segregation of components through eluting with solvent combinations of varying polarities. In vacuum, all the fractions of the column were extracted and concentrated. Spectroscopic approaches for example GC-MS, NMR and FT-IR were used to determine the precise molecular structure of the isolated bioactive molecule.

Larvicidal activity: Larvicidal activities of 25, 50, 75 and 100 µg/mL of plant powder, ethanolic extract and isolated compound **1** were screened as previously described [31]. Commercial pesticide permethrin was used as positive control. The 50% lethal doses (LD_{50}) values of the compounds were calculated using probit analysis and statistically analyzed using SPSS version 16.0 software.

Molecular docking: AutoDock Vina 1.1.2 was used to analyze the relationships and binding modes among the isolated component **1**, permethrin and the odorant-binding proteins (OBP) of mosquito *C. quinquefasciatus* [32]. The Protein Data Bank (http://www.rcsb.org) is the source for the crystal structure of OBP (PDB ID: 3OGN). Isolated chemical **1** and permethrin's 3D structures were calculated using ChemDraw Ultra 12.0 and Chem3D Pro 12.0. AutoDock Tools 1.5.6 was employed to construct the input files for AutoDock Vina. The grid coordinates were set at (*x*: 18.681), (*y*: 49.66) and (*z*: 11.409), with size (*x*: 22), (*y*: 20) and (*z*: 22) and spacing 1.0 Å, respectively. The level of thoroughness was set to 8. The other Vina docking defaults were left alone. The Discovery Studio 2019 software was used to graphically assess the findings and found that the molecule with the lowest binding affinity was the most effective [31-34].

Statistical analysis: The larvicidal activity data was analyzed through probit analysis using SPSS (v 16.0). The values are the means of three replicates ± SD.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis: Table-1 displays the findings of a preliminary phytochemical analysis performed on medication powder and ethanolic extract of plant. *Acalypha indica* ethanolic extract contains flavonoids, alkaloids, steroids, saponins and sugars. The ethanolic extract of *Acalypha indica* lacks the quinone and tannin found in the plant powder.

TABLE-1
PRELIMINARY PHYTOCHEMICAL SCREENING

Test for	Plant powder	Ethanol
Flavanoids	Presence	Presence
Lignins	Absence	Absence
Alkaloids	Presence	Presence
Terpenoids	Absence	Absence
Saponins	Presence	Presence
Quinone	Presence	Absence
Tannins	Presence	Absence
Coumarin	Absence	Absence
Steroids	Presence	Presence
Sugar	Presence	Presence

Characterization of isolated bioactive compound: The structure postulated for isolated compound **1** is in excellent agreement with the spectral data (GC-MS, ^{1}H NMR, ^{13}C NMR and FT-IR). According to the FT-IR spectra, the -Ph, -CH_2 and -C=O groups having prominent bands at 3245.55, 2978.74 and 1725.70 cm^{-1} (Fig. 1). According to ^{1}H NMR spectroscopy, the Ph, CH_2 and CH_3 protons were detected by peaks at δ 6.9-7.3, 4.6 and 0.9 ppm. The C=O, CH_2 and CH_3 carbon atoms are represented by signals at δ 201.52, 66.9 and 11.6-14.1 ppm in the ^{13}C NMR spectra. The molecular weight of compound **1** was in good agreement with the mass spectral data, which were backed by expected target compound.

***Bis*(2-ethylhexyl) phthalate (1):** Brown solid; *m.w.*: 390.56; m.p.: 245 ºC; IR (KBr, ν_{max}, cm^{-1}): 3245.55 (Ph-CH *str.*), 2978.74 (CH_2), 2935.74 (CH_3), 1725.70 (C=O), 1092.10 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.9-7.3 (4H, m, phenyl ring), 4.6 (4H, s, -CH_2), 1.2-2.3 (16H, m, -CH_2), 2.7 (2H, m, -CH), 0.9 (12H, s, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 201.52 (2C, C=O), 128.06, 127.53, 124.65, 119.85 (6C, Ph), 66.9 (2C, CH_2), 39.6 (2C, CH), 30.8 (2C, CH_2), 29.3 (2C, CH_2), 23.7 (2C, CH_2), 23.0 (2C, CH_2), 14.1 (2C, CH_3), 11.6 (2C, CH_3). GC-MS: *m.w.*: 390.56; *m/z* 390.00 (100%); Elemental analysis (%): calcd. (found) for $C_{24}H_{38}O_4$: C, 73.81 (73.83); H, 9.81 (9.76).

Larvicidal activity: Larvicidal efficacy towards *Culex quinquefasciatus* second instar mosquito larvae was tested using plant powder, ethanolic extract, isolated component **1** and the commercial insecticide permethrin. The isolated compound displayed significant larvididal activity (LD_{50}: 38.23 µg/mL) than control permethrin (LD_{50}: 54.05 µg/mL) ethanolic extract and plant powder. The plant powder (LD_{50}: >100 µg/mL) and ethanolic extract (LD_{50}: 75.13 µg/mL) revealed moderate larvicidal effect towards *C. quinquefasciatus* (Table-2).

Docking: The Autodock Vina software was employed to examine the docking performance of the isolated compound **1**, control permethrin with protein 3OGN. Compound **1** demonstrates better binding affinity (-7.9 kcal/mol) to 3OGN protein than the control drug permethrin, which has a binding affinity of (-6.8 kcal/mol). Protein-ligand bonding is stable because of hydrogen bonding and the ideal bond distance between the H-acceptor and H-donor atoms is less than 3.5 Å. The isolated compound **1** and the control drug permethrin both had hydrogen bond distances less than 3.5 Å, which indicates strong hydrogen bonding. Compound **1** does not create any hydrogen bond with the receptor 3OGN. The amino acids Leu15, Ala18, Leu19, Leu58, Leu76, Leu80, Ala88, Met91, His111, Trp114, Tyr122, Phe123 and Leu124 were associated in hydrophobic contacts. With the receptor 3OGN, the control permethrin does not create any hydrogen bonds. Hydrophobic interactions involving the amino acids Leu15, Leu19, Phe59, Leu73, Leu76, His77, Leu80, Ala88, Met89, Gly92, Phe123 and Leu124. Figs. 2 and 3 demonstrate the hydrophobic and hydrogen bond associ-

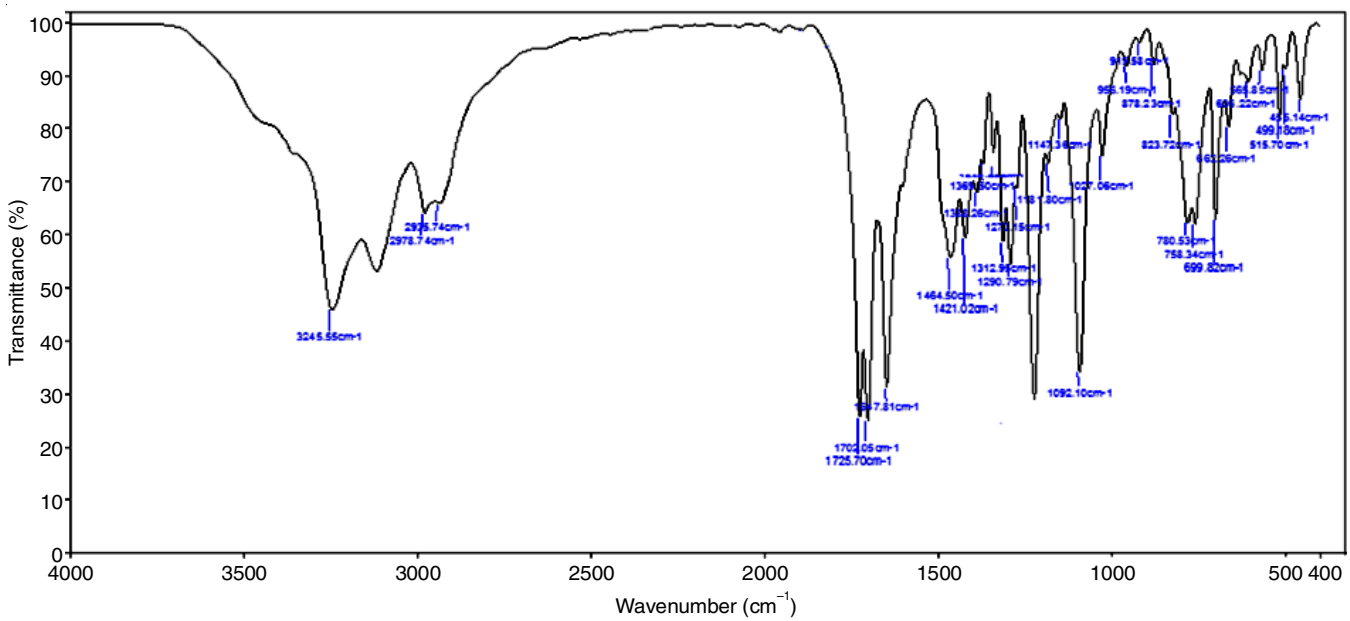

Fig. 1. FT-IR spectrum of the isolated compound **1**

TABLE-2
LARVICIDAL ACTIVITY

Compound	Mortality (%)/Concentration (µg/mL)[#]				LD_{50}
	25	50	75	100	
Plant powder	18 ± 0.32	31 ± 0.10	36 ± 0.53	42 ± 0.65	>100
Ethanolic extract	26 ± 0.32	38 ± 0.10	50 ± 0.53	58 ± 0.65	75.13
Isolated compound	40 ± 0.81	53 ± 0.87	68 ± 0.35	82 ± 0.65	38.23
Permethrin	31 ± 0.39	45 ± 0.32	53 ± 0.11	74 ± 0.22	54.05

[#]Value were the means of three replicates ± SD.

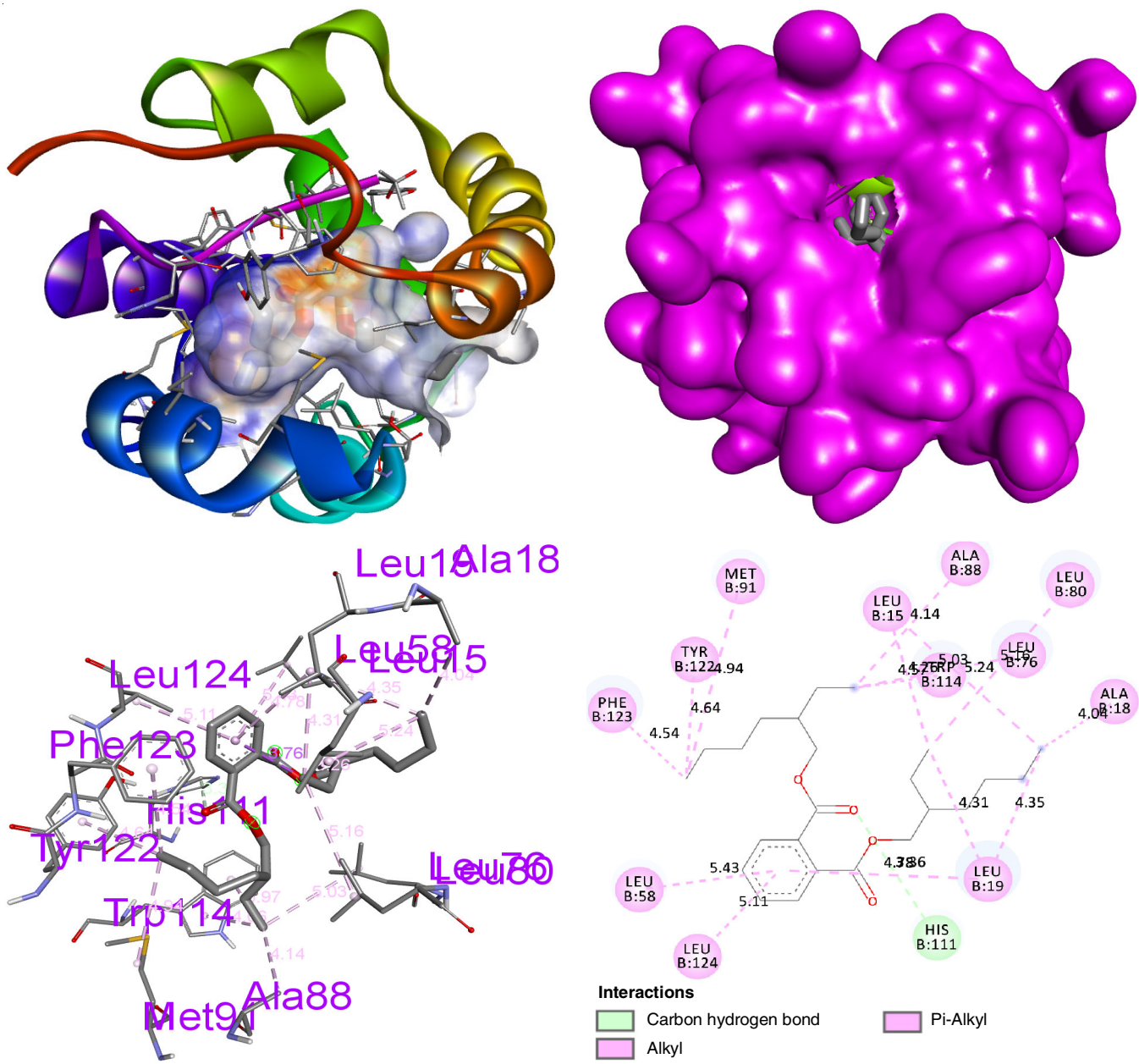

Fig. 2. Interactions of isolated compound **1** within the binding pocket of 3OGN receptor

ations in 3OGN receptor with compound **1** and control permethrin. The results (Table-3) show that compound **1**, as compared to the standard treatment of permethrin, significantly reduces mosquito OBP activity.

TABLE-3
MOLECULAR DOCKING RELATIONS OF
COMPOUND **1** AND CONTROL PERMETHRIN

Compounds	Mosquito odorant-binding protein 3OGN		
	Binding affinity (kcal/mol)	No. of H-bonds	H-bonding residues
1	-7.9	0	–
Permethrin	-6.1	0	–

Conclusion

The current study concludes that an ethanolic extract of the medicinal plant *Acalypha indica* L. leaves was subjected to pre-liminary phytochemical screening, bioactive fraction extra-ction, larvicidal activity and molecular docking research. The preliminary phytochemical analysis of *Acalypha indica* ethanolic extract demonstrates the existence of flavonoids, alkaloids, saponins, steroids and sugars. GC-MS, NMR (^{1}H & ^{13}C), FT-IR and elemental analysis validated the bioactive component found in the ethanolic extract of *Acalypha indica* was *bis*(2-ethylhexyl)phthalate (**1**). Compound **1** displayed more larvicidal activity than plant powder and ethanolic extract, with an LD_{50} of 38.23 µg/mL, which was superior to the LD_{50} of the control permethrin, which was 54.05 µg/mL. The LD_{50} values for plant powder and ethanolic extract against *Culex quinque-fasciatus* were >100 and 75.13 µg/mL, respectively. The molecular docking investigations provide substantial support for larvicidal action.

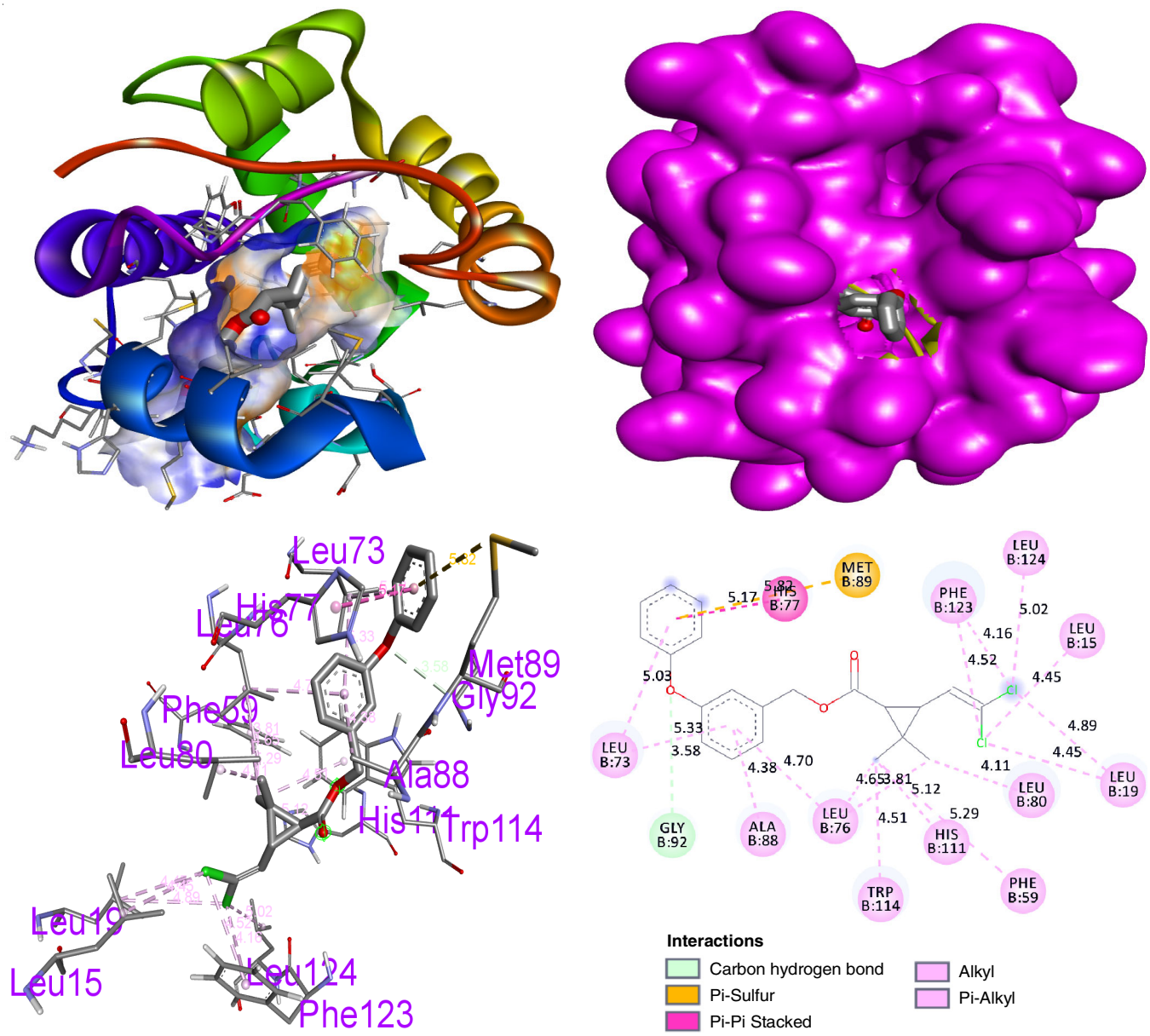

Fig. 3. Interactions of control permethrin within the binding pocket of 3OGN receptor

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. D.C. Atkinson and R. Hicks, *Br. J. Pharmacol.*, **41**, 480 (1971); https://doi.org/10.1111/j.1476-5381.1971.tb08045.x
2. M.S. Baliga, G.C. Jagetia, J.N. Ulloor, M.P. Baliga, P. Venkatesh, R. Reddy, K.M. Rao, B.S. Baliga, S. Devi, S.K. Raju and V. Veeresh, *Toxicol. Lett.*, **151**, 317 (2004); https://doi.org/10.1016/j.toxlet.2004.01.015
3. R.N. Okigbo and O.D. Omodamiro, *J. Herbs Spices Med. Plants*, **12**, 117 (2007); https://doi.org/10.1300/J044v12n01_11
4. C.X. Liu, *J. Ethnopharmacol.*, **19**, 119 (1987); https://doi.org/10.1016/0378-8741(87)90035-3
5. B. Desta, *J. Ethnopharmacol.*, **39**, 129 (1993); https://doi.org/10.1016/0378-8741(93)90028-4
6. C. Anesini and C. Perez, *J. Ethnopharmacol.*, **39**, 119 (1993); https://doi.org/10.1016/0378-8741(93)90027-3
7. A. Nick, T. Rali and O. Sticher, *J. Ethnopharmacol.*, **49**, 147 (1995); https://doi.org/10.1016/0378-8741(95)01315-6
8. R. Perumal Samy, S. Ignacimuthu and D.P. Raja, *J. Ethnopharmacol.*, **66**, 235 (1999); https://doi.org/10.1016/S0378-8741(99)00038-0
9. A. Alcorta, A. Porta, A. Tárrega, M. Dolores Alvarez and M. Pilar Vaquero, *Foods*, **10**, 293 (2021); https://doi.org/10.3390/foods10020293
10. S.P. Hiremath, K. Rudresh, S. Badami, S.B. Patil and S.R. Patil, *J. Ethnopharmacol.*, **67**, 253 (1999); https://doi.org/10.1016/S0378-8741(98)00213-X

11. A. Shirwaikar, K. Rajendran, R. Bodla and C.D. Kumar, *J. Ethnopharmacol.*, **94**, 267 (2004); https://doi.org/10.1016/j.jep.2004.05.010
12. J.S. Reddy, P.R. Rao and M.S. Reddy, *J. Ethnopharmacol.*, **79**, 249 (2002); https://doi.org/10.1016/S0378-8741(01)00388-9
13. C. Mohan, S. Dinakar, T. Anand, R. Elayaraja and B. Sathiya Priya, *Int. J. Pharm. Tech. Res.*, **4**, 1050 (2012).
14. A.K. Das, F. Ahmed, N.N. Biswas, S. Dev and M.M. Masud, *Dhaka Uni. J. Pharm. Sci.*, **4**, (1970); https://doi.org/10.3329/dujps.v4i1.204
15. M. Govindarajan, A. Jebanesan, D. Reetha, R. Amsath, T. Pushpanathan and K. Samidurai, *Eur. Rev. Med. Pharmacol. Sci.*, **12**, 299 (2008).
16. C. Krishnaraj, P. Muthukumaran, R. Ramachandran, M.D. Balakumaran and P.T. Kalaichelvan, *Biotechnol. Rep.*, **4**, 42 (2014); https://doi.org/10.1016/j.btre.2014.08.002
17. M. Rahmatullah, S. Hossan, A. Khatun, S. Seraj and R. Jahan, *Malar. Res. Treat.*, **2012**, 1 (2012); https://doi.org/10.1155/2012/371798
18. G. Benelli, A. Lo Iacono, A. Canale and H. Mehlhorn, *Parasitol. Res.*, **115**, 2131 (2016); https://doi.org/10.1007/s00436-016-5037-y
19. S.V. Mayer, R.B. Tesh and N. Vasilakis, *Acta Trop.*, **166**, 155 (2017); https://doi.org/10.1016/j.actatropica.2016.11.020
20. K.S. Hwang, Y.K. Kim, K.W. Park and Y.T. Kim, *Pest Manag. Sci.*, **73**, 1564 (2017); https://doi.org/10.1002/ps.4580
21. P.C. Bezerra-Silva, K.A. Dutra, G.K. Santos, R.C. Silva, J. Iulek, P. Milet-Pinheiro and D.M. Navarro, *PLoS One*, **11**, e0150008 (2016); https://doi.org/10.1371/journal.pone.0150008
22. A. Bazáes, J. Olivares and O. Schmachtenberg, *J. Chem. Ecol.*, **39**, 451 (2013); https://doi.org/10.1007/s10886-013-0268-1
23. C.A. de March and J. Golebiowski, *Biochimie*, **107**, 3 (2014); https://doi.org/10.1016/j.biochi.2014.06.006
24. M. Pechlaner and C. Oostenbrink, *J. Chem. Inf. Model.*, **55**, 2633 (2015); https://doi.org/10.1021/acs.jcim.5b00673
25. E.A. Sofowara, Medicinal Plant and Traditional Medicine in Africa, Spectrum Books, Ibadan, Nigeria, edn. 2, p. 289 (1993).
26. J.B. Herborne, Phytochemical methods. A Guide to Modern Techniques of Plant Analysis, vol. 2, p. 5 (1973).
27. D.E. Okwu, *Glob. J. Pure Appl. Sci.*, **7**, 455 (2001); https://doi.org/10.4314/gjpas.v7i3.16293
28. T. Rahila, N. Rukhsandra, A.A. Zaidi and R. Shamshila, *Pak. Vet. J.*, **14**, 160 (1994).
29. P. Houghton and A. Raman, Laboratory Handbook for the Fractionation of Natural Extracts, Springer Science & Business Media (2012).
30. M. Ebadi, Pharmacodynamic Basis of Herbal Medicine, CRC Press (2006).
31. S. Tennyson, K.J. Ravindran and S. Arivoli, *Asian Pac. J. Trop. Biomed.*, **2**, S1130 (2012); https://doi.org/10.1016/S2221-1691(12)60372-4
32. O. Trott and A.J. Olson, *J. Comput. Chem.*, **31**, 455 (2010); https://doi.org/10.1002/jcc.21334
33. G. Dayana Jeyaleela, N. Balasubramani and J. Rosaline Vimala, *Asian J. Pharm. Clin. Res.*, **12**, 110 (2019); https://doi.org/10.22159/ajpcr.2019.v12i2.28384
34. G. Dayana Jeyaleela, J. Rosaline Vimala, S. Senthil and K. Manjula, *Asian J. Chem.*, **31**, 2632 (2019); https://doi.org/10.14233/ajchem.2019.22217