INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Extraction, identification and molecular docking studies evaluation of *Momordica tuberosa* (Roxb) against Anopheles

Muthumani Mamudha[1]*, Anbu Jeba Sunilson[2]

1 Research scholar, Department of siddha medicine, Tamil university, Thanjavur, India
2 Associate professor, Department of siddha medicine, Tamil university, Thanjavur, India

OPEN ACCESS

Received: 27.05.2021
Accepted: 16.08.2021
Published: 22.09.2021

Citation: Mamudha M, Sunilson AJ (2021) Extraction, identification and molecular docking studies evaluation of *Momordica tuberosa* (Roxb) against Anopheles. Indian Journal of Science and Technology 14(31): 2550-2556. https://doi.org/10.17485/IJST/v14i31.913

* **Corresponding author**.

mamudha2014@gmail.com

Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: This study aimed to identify phytochemicals from *Momordica tuberosa* (*M. tuberosa*) leaves and it's in silico larvicidal potential compounds against Anopheles. Further, to analyze their inhibitory mechanisms against the Anopheles angiotensin-converting enzyme (ACE). In view of the above fact, we made an attempt to discover potential and novel natural mosquito larvicidal compounds with least impact on human health. **Method:** The GC-MS analysis confirmed the phytoconstituents from the methanol extracts of *M. tuberosa* through GC-MS and selected compounds were submitted to docking studies on Anopheles angiotensin-converting enzyme as possible targets. **Findings**: GC-MS analysis of the extract showed the presence of 20 compounds with Limonene dioxide, Neophytadiene and Palmitic acid as main constituents. Three Selected compounds (Limonene dioxide, Neophytadiene and palmitic acid) specifically binds the binding site of an angiotensin-converting enzyme (ACE). The binding energy Limonene dioxide (- 6 kcal/mol), Neophytadiene (- 6.5 kcal/mol), Palmitic acid (- 5.7 kcal/mol) and interaction were analyzed. The present study displays better docking binding energy in the selected compounds. The results further substantiate that the 3 compounds of the M. tuberose found to be model candidates for the design and development of potential larvicidal. **Novelty:** The study helps to pave the first way to develop the promising larvicidal compound from plants. This result visibly suggests that compounds (Limonene dioxide, Neophytadiene and Palmitic acid) from *M. tuberosa* could act as potential larvicidal agents – to control Anopheles.

Keywords: Phytochemical; Molecular docking; anangiotensinconverting enzyme (ACE); larvicidal; Chromatography

1 Introduction

Malaria is one of the most complicated life-threatening infections caused by parasites that are transferred to people by mosquito bites from infected Anopheles mosquitoes. Both genders are affected, resulting in serious health and social consequences. In 2020, the World Health Organization (WHO) projected that there were 228 million cases of malaria distributed over 87 countries. Malaria claimed the lives of 405,000 people

in 2018 [1]. The Anopheles mosquito is one of the most common malaria vectors. Infection begins with a bite from a female mosquito carrying the parasite, which is then transported - in circulatory system and eventually to the liver, where it matures and reproduces [2]. Malaria control has spawned a slew of new techniques and strategies. Eliminating the vector by environmental changes, biological control, the use of long-lasting insecticidal nets (LLINs), and indoor residual spraying is one of the long-lasting insecticidal nets (IRS). Another effective method is to remove mosquitoes while they are still in the larval stage by stopping them from developing. Organophosphates and carbamates are two types of synthetic insecticides that inactivate anangiotensin-converting enzyme (ACE) and are now used to treat malaria. However, their use raises health concern about the living system's and the environment's safety [3]. In order to protect human health, the use of safe, natural chemicals derived from plant extracts has become a viable alternative to synthetic insect repellents. The use of traditional plants, different parts and their various products in the control of mosquitoes has been well established globally by numerous researchers [4]. The larvicidal properties of phytochemical components of plants have been documented in India along with the larvicidal and inhibiting growth hormone activity [5]. Identification of the insecticide target is important to control the mosquitoes [6]. The angiotensin-converting enzyme, ACE important for larval development and the egg-laying process [7]

An ACE is important for the mosquito development cycle, so the researchers develop the inhibitors against these particular targets [8]. The AnoACE contain 598 residues and AnoACE2 has six potential N-glycosylation sites (residues 69, 74, 106, 187, 212 and 327). ACE inhibitor fosinoprilat of the binding site revealed a large region of electron density located adjacent to the zinc ion of the complex structure His353, His383 and Tyr523 Gln281, Lys511 and Tyr520. This level of detailed knowledge makes AnoACE2 an attractive target for applying structure-based drug design to developing potent and, importantly, highly selective inhibitors which could be used as insecticides. Thus, for the development of inhibitors of practical value in killing mosquito larvae, it is important to design new compounds that are both potent and highly selective.

Asian traditional plant *Momordica tuberosa* (Roxb) vine of Momordica genus has been used in various medical systems for a long time [9]. *Momordica tuberosa* (*M. tuberosa*) is commonly known as or Athalakkai (Tamil), Karchikai (Kannada), and Kasarakayee (Andhra Pradesh) in India.

M. tuberosa, the considered medicinal plant, produces different types of secondary metabolites (phytochemicals, essential oil, and fatty acids). These valuable bioactive properties prevent diseases and maintain their functions and homeostasis [10].

The present study aimed to investigate the chemical composition and understand the molecular mechanism of bioactive compounds that binds to the active site of the angiotensin-converting enzyme, ACE [8].

2 Methodology

2.1 Plant material collection

The leaves of *M. tuberosa* were collected from Virudhunagar District, Tamil Nadu, India. The collected voucher specimen (Acc. No.: GUD 0923) was authenticated by Botanist from the department of Biology of the Gandhi gram rural institute. Dindigul District, Tamil Nadu, India.

2.2 Extraction of *Momordica tuberosa*

The leaves of *M. tuberosa* was shadow dried and coarsely powdered and soaked in methanol for six days respectively in the sterilized flask. After the incubation filter, the solvent extract and the extract were evaporated and dried under vacuum using a rotary evaporator at 60-70 °C. The residues were weighed and preserved at 4 °C until use [11].

2.3 GC-MS (Gas Chromatography-Mass Spectrometry) analysis

M. tuberosa extract was subjected to column chromatography to isolate the active constituents as reported before. The methanol extract was tested for alkaloids, flavonoids, phenols, steroids, tannins, terpenoids, cardiac glycosides, saponins, reducing sugars, volatile oils, carbohydrates, and protein/amino acid [12–22]. The phytochemical investigation of methanolic extract was performed on a GC-MS equipment SHIMADZU model GCMS-QP-2010 plus. Experimental conditions of the GC-MS system were as follows: RT 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.32 mm, Film thickness: 0.50μm. The flow rate of the mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature-programmed (oven temperature) of 40°C was raised to 260°C at 5°C/min and the injection volume was 1μl. Samples dissolved in methanol were run fully at a range of 10-350 m/z and the results were compared by using Wiley Spectral library search programme.

2.4 In silico virtual screening studies

Molecular docking helps to analyze the molecular interaction of drugs and protein to the enzyme pocket. The crystal structure of the AnoACE2 (PDB ID: 6S1Z) was applied for the docking study[23]. Computer-aided virtual screening was carried out with three ligand compounds against the binding site of AnoACE2 using PyRx which incorporates Auto Dock and Auto Dock Vina, was used for virtual screening of ligands, with the Lamarckian genetic algorithm (LGA) as the scoring function. To dock the ligands, the active site dimensions were adjusted to a grid size of Centre X=64.31, Centre Y=40.70, and Centre Z=84.94 (cover the whole protein), with 10 maximum exhaustiveness determined for each ligand. To analyze the docking calculations, 10 conformers are considered for each protein-target ligand and the lowest binding energy is selected to identify the best binding complex. The 2D and 3D molecular interaction models of the docked complex involving displayed using Accelrys Discovery Studio Visualizer software version 3.5.

3 Results and discussion

3.1 Compounds Isolation

The methanolic extract sample was run through GCMS chromatography for the identification of pure compounds (Figure 1). The phytochemical screening of the methanol extracts of *M. tuberosa* revealed the presence of phytoconstituents in our previous work. This is existing, so we proceed with the analysis of phytoconstituents using GC-MS Liquid Chromatography-Mass Spectrometry (GC-MS) studies. The Table 1 shows that 20 compounds were detected from the methanol extracts of *M. tuberosa* using GC-MS analysis. GC-MS analysis of methanol extracts of *M. tuberosa* leaves had detected three peaks with the retention time 10.962, 12.629, and 13.992 minutes (Figure 2 A, B& C). The GC-MS spectrums interpretation was performed using an inbuild GCMS library application.

Three compounds (Limonene dioxide, Neophytadiene and Palmitic acid) selected for further molecular interaction study because these are major compounds in *M. tuberosa* extract. Mater H.Mahnashi group also identified 22 compounds from Moringa oleifera using GC-MS method after identification molecular docking with alpha amylase enzymes and explains synergetic effects of compounds against amylase.[24]

Table 1.

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	9.742	1054934	0.69	544589	1.08	1.94	3-Octadecene,(E)-(CAS)
2	10.400	949377	0.62	219408	0.43	4.33	1-hydroxylinalool
3	10.962	931579	0.61	310130	0.61	3.00	Limonenedioxide1
4	11.787	1957024	1.27	641062	1.27	3.05	Tetradecanoicacid(CAS)Myristicacid
5	12.088	1171760	0.76	578181	1.15	2.03	3-Eicosene,(E)-(CAS)
6	12.629	2196562	1.43	971912	1.93	2.26	Neophytadiene
7	12.726	930659	0.61	404308	0.80	2.30	2-Pentadecanone,6,10,14-trimethyl-(CAS)6,
8	12.901	1085023	0.71	398413	0.79	2.72	9-Octadecenoicacid(Z)-(CAS)Oleicacid
9	13.109	931850	0.61	376992	0.75	2.47	9-Eicosyne(CAS)
10	13.560	2995591	1.95	1371977	2.72	2.18	Pentadecanoicacid,14-methyl-,methylester(
11	13.992	73401041	47.74	25304204	50.15	2.90	Hexadecanoicacid(CAS)Palmiticacid
12	14.125	2578602	1.68	589955	1.17	4.37	2-Propenoicacid,2-methyl-,2-[(1,1-dimethyle
13	14.233	1365304	0.89	554779	1.10	2.46	9-Eicosene,(E)-(CAS)
14	15.437	968622	0.63	399689	0.79	2.42	9,12,15-Octadecatrienoicacid,methylester,(Z
15	15.554	14202181	9.24	5353649	10.61	2.65	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-
16	15.621	1662240	1.08	732668	1.45	2.27	Octadecanoicacid,methylester(CAS)Methy
17	15.829	7378013	4.80	1729640	3.43	4.27	Oxacycloheptadec-8-en-2-one(CAS)Ambrett
18	15.907	11745173	7.64	2971944	5.89	3.95	9,12,15-Octadecatrienoicacid,methylester,(Z
19	16.070	23401759	15.22	6506909	12.90	3.60	Octadecanoicacid(CAS)Stearicacid
20	21.956	2837937	1.85	494417	0.98	5.74	Hexadecanoicacid,2-hydroxy-1-(hydroxymet

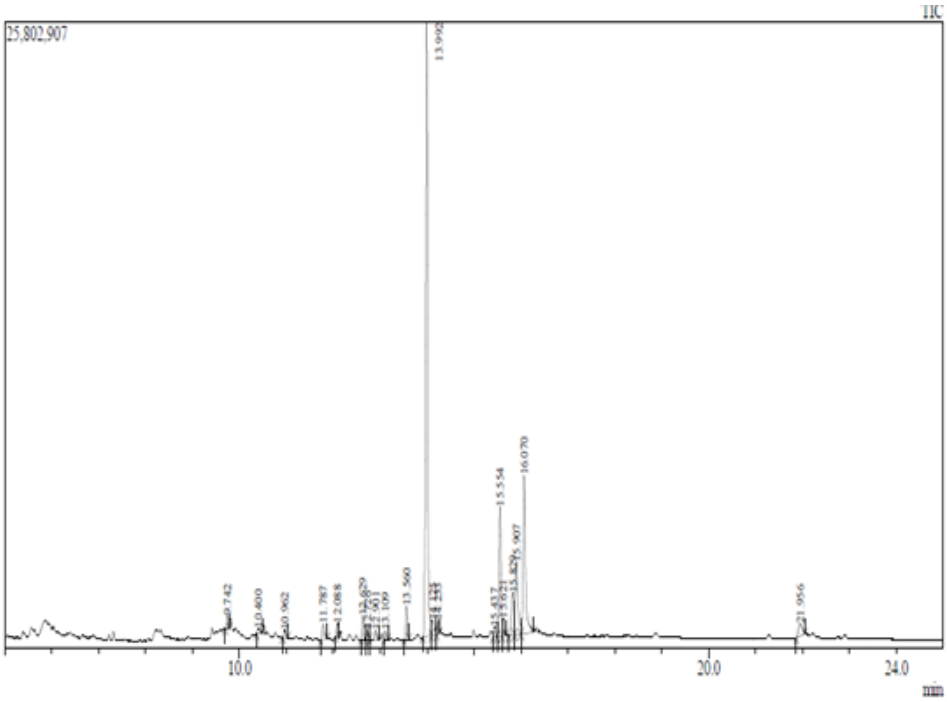


Fig 1. A - GC-MS spectrums interpretation

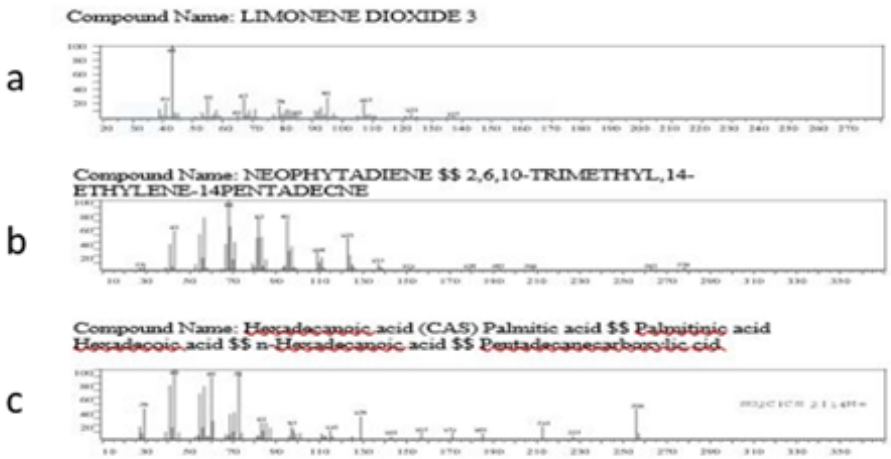


Fig 2. a, b, c - GCMS spectrum interpretation

Molecular docking studies

The result gives a value of 77.36%, from which it may be inferred that this model is of good quality. The results show that the amino acid residues Gln281, Lys511 and Tyr520, His 383, His 389, and Glu 411 are the active site residues that are responsible for binding to the ligand molecules. Our study involves docking of Limonene dioxide, Neophytadiene, Palmitic acid with Ano ACE is using the PyRx virtual screening tool. Nine conformers were considered for each ligand-enzyme complex, and the lowest docking energy was chosen to identify the preferable binding mode[25] of the docked compound in AnoACE as in (Figure 3). The molecular docking interaction models of the two ligand molecules and the target enzyme were illustrated in (Figure 3A, B & C). The molecular interaction results like H-bonds, π-π, and Pi-sigma are represented in (Figure 3A, B &

C). The binding energy is corresponding to Limonene dioxide (- 6 kcal/mol), Neophytadiene (- 6.5 kcal/mol), Palmitic acid (- 5.7 kcal/mol), and comparatively Neophytadiene shown better binding to AnoACE enzyme. These bioactive compounds specifically bind to the active site of the enzyme and it may inhibit the enzyme action so it's directly inhibited the disease-causing mosquito development. This kind of study, phytochemical against enzyme inhibition done by various groups, Nwakulite groups describe the co ligands inhibits the amylase activity and control the glucose metabolism[26]. Fatema R. Saber group studied the phytochemical interaction with active site amino acid residue of BChE and ACE enzymes by a mixture H-bonding, and hydrophobic forces[27]. The AnoACE2 is an important biological growth enzyme for mosquito. The particular AnoACE2 is an attractive target for the development of the larvicidal compounds.

The Molecular docking study additionally gives information about molecules binding to active site AnoACE2. These studies provide scientific evidence to structure-based drug design against mosquito larvae.

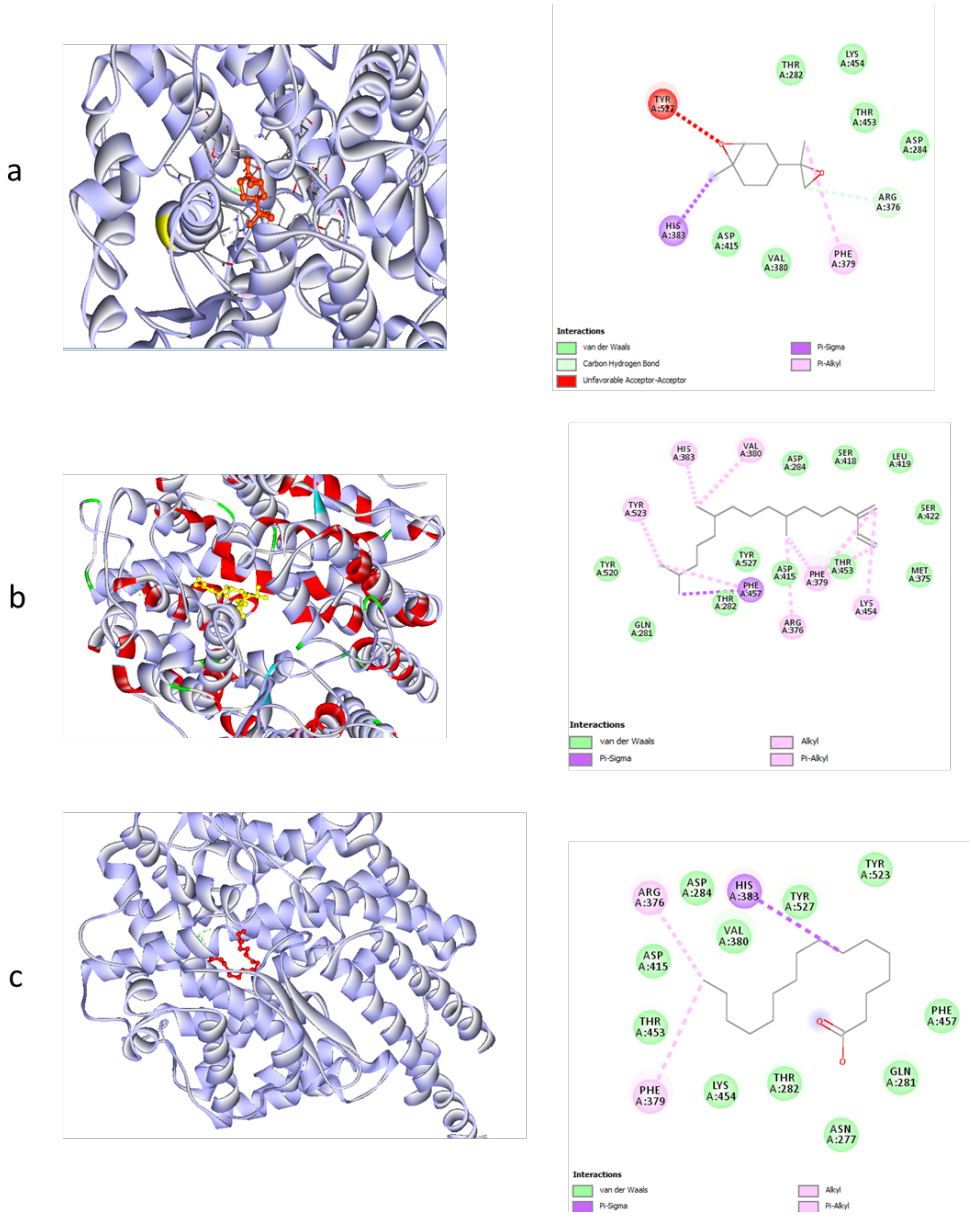
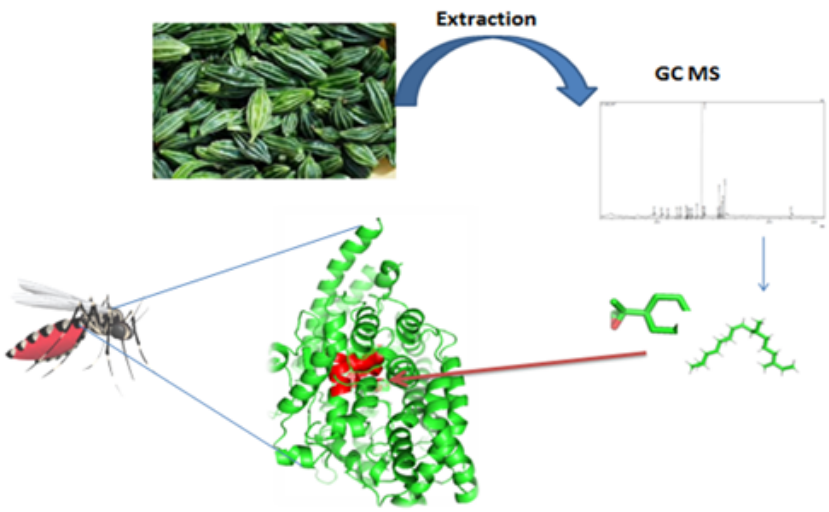


Fig 3. A- AnoACE Limonene dioxide, B- Ano ACE Neophytadiene, C - Ano ACE Palmitic acid

Graphical abstract



4 Conclusion

M. tuberosa is a plant material form a new source of phytoconstituents that has an important insecticidal activity for mosquito control. The present study demonstrated that the in silico molecular docking interactions of the phytoconstituents and AnoACE showed good binding energy. There is a strong correlation between the results of molecular docking and the biological effects which suppose that the tested compounds may be useful for designing new and safe inhibitors against AnoACE enzyme. The computed binding energy of Limonene dioxide, Neophytadiene, Palmitic acid shows these compounds are lethal to Anopheles larvae development. In future development of a resistance system, Limonene dioxide, Neophytadiene, Palmitic acid compounds could be used as larvicides as they are found as a safe and efficient alternative for mosquito larval control in upcoming years.

References

1) WHO. World Malaria Report. 2019. Available from: https://www.who.int/publications-detail/world-malaria-report-2019.
2) Venugopala KN, Gleiser RM, Chalannavar RK, Odhav B. Antimosquito properties of 2-substitutedphenyl/benzylamino-6-(4-chlorophenyl)-5-methoxycarbonyl-4-methyl-3,6-dihydropyrimidin-1-iumchlorides against Anopheles arabiensis. *Med Chem*. 2014;10:211–219.
3) Mohankumar TK, Shivanna KS, Achuttan VV. Screening of Methanolic Plant extracts against larvae of Aedes aegypti and Anopheles stephensi in Mysore. *J Arthropod Borne Dis*. 2016;10:303–314.
4) Gaddaguti V, Rao TV, Rao AP. Allu Prasada Rao Potential mosquito repellent compounds of Ocimum species against 3N7H and 3Q8I of Anopheles gambiae. *3 Biotech*. 2016;6(26). Available from: https://doi.org/10.1007/s13205-015-0346-x.
5) Tzotzos G, Iley JN, Moore EA. MooreNew insights on repellent recognition by Anopheles gambiae odorant-binding protein . *PLOS ONE*. 2020. Available from: https://doi.org/10.1371/journal.pone.0194724.
6) Javidfar M, Ahmadi S. QSAR modelling of larvicidal phytocompounds against Aedes aegypti using index of ideality of correlation. *SAR and QSAR in Environmental Research*;31(10):717–739. Available from: https://doi.org/10.1080/1062936X.2020.1806922.
7) Hussein MA, Zyaan OH, bakr H Abdel Monsef A, Rizk SA, Farag SM, Hafez SE, et al. Synthesis, molecular docking and insecticidal activity evaluation of chromones of date palm pits extract against Culex pipiens(Diptera: Culicidae). *International Journal of Mosquito Research*. 2018;5(4):22–32. Available from: http://www.dipterajournal.com/pdf/2018/vol5issue4/PartA/5-3-18-682.pdf.
8) Hasan ZIA, Williams H, Ismail NM, Othman H, Cozier GE, Acharya KR, et al. The toxicity of angiotensin converting enzyme inhibitors to larvae of the disease vectors Aedes aegypti and Anopheles gambiae . *Scientific Reports*. 2017;7. Available from: https://doi.org/10.1038/srep45409.
9) Gopalasatheeskumar K. An Updated Pharmacological Overview On Momordica cymbalaria (Athalakkai). *Innoriginal International Journal of Sciences*. 2018;5(1):28–31.
10) Louis LL, Anna-Bella FA. Vector biology prospects in dengue research. *Memorias do Instituto Oswaldo Cruz*. 2012;107(8):1080–1082. Available from: https://doi.org/10.1590/S0074-02762012000800022.
11) Sheila BA, David PT, Armanda D, Bastos S, Rosemary S. Assessment of risk of dengue and yellow fever virus transmission in three major Kenyan cities based on Stegomyia indices. *Plos Neglected Tropical Diseases*. 2017;11(8):1–20. Available from: https://doi.org/10.1371/journal.pntd.0005858.
12) Madani AT, Al-Mazrou YY, Al-Jeffri MH, Mishkhas AA, Al-Rabeah AM, Turkistani AM, et al. Rift valley fever epidemic in Saudi arabia: epidemiological, clinical and laboratory characteristics. *Clinical Infectious Diseases*. 2003;37(8):1084–1092. Available from: https://doi.org/10.1086/378747.
13) Brady OJ, Godfray HCJ, Tatem AJ, Gething PW, Cohen JM, McKenzie FE, et al. Vectorial capacity and vector control: Reconsidering sensitivity to parameters for malaria elimination. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2016;110(2):107–117. Available from:

https://doi.org/10.1093/trstmh/trv113.
14) Rattanam AR, Fatma ZW, Maniam T, Sreeramanan S, and SJJ. Larvicidal efficacy of different plant parts of railway creeper Ipomoea cairica extract against Dengue vector Mosquito, Aedes albopictus (Diptera: Cuilicidae) and Aedes aegypti (Diptera: Cuilicidae). *Journal of Insect Science*. 2014;14(1):1–6. doi:10.1093/jisesa/ieu042.
15) Khalid A, Saeed HS, Ibrahim N, Al-KhuraijiIbrahim A, Abir AZAS, Naimah AA, et al. Larvicidal efficacy of ethanolic leaf extracts of four selected local plants from hail region, northern Saudi Arabia, against the dengue fever vector, Aedes aegypti (L.) under laboratory conditions. *International Journal of Mosquito Research*. 2017;4(3):81–87.
16) Lemine AMM, Lemrabott MAO, Ebou MH, Lekweiry KM, Salem MSOA, Brahim KO, et al. Mosquitoes (Diptera: Culicidae) in Mauritania: a review of their biodiversity, distribution and medical importance. *Parasites & Vectors*. 2017;10(35):1–13. Available from: https://doi.org/10.1186/s13071-017-1978-y.
17) El-Assar A. Response of (Zaghloul) date yield and fruit characteristics to various organic and inorganic fertilization types as well as fruit thinning models in a rich carbonate soil. *Journal of Agricultural sciences Mansoura University*. 2005;30(5):2795–2814.
18) Mohammad MH, Mostafa MW, Vandita VS, Sequeira V, shafiur MRM. Chemical composition of date pits and its potential for developing value added product a review. *Polish Journal of Food Nutrition Science*. 2014;64(4):215–226.
19) Chapagain B, Larvicidal ZW. Larvicidal effects of aqueous extracts of Balanites aegyptica(dessert date) against the larvae of Culex pipiens mosquitoes. *African Journal of Biotechnology*. 2005;4:1351–1354. Available from: https://www.ajol.info/index.php/ajb/article/view/71379.
20) Wiesman Z, Chapagain BP. Larvicidal activity of saponin containing extracts and fractions of fruit mesocarp of Balanites aegyptica. *Filoterapia*. 2006;77(6):420–424. Available from: https://doi.org/10.1016/j.fitote.2006.05.012.
21) Elamin MM, Satti AA. Insecticidal potentialities of Balanites aegypticaextracts against the Khapra beetle (Trogoderma granarium). *Global Advanced Research Journal of Environmental Science and Toxicology*. 2013;2(1):5–10. Available from: http://garj.org/garjest/1/2013/2/1/insecticidal-potentialities-of-balanites-aegyptiaca-extracts-against-the-khapra-beetle-trogoderma-granarium.
22) Hoda SM, Fahmy MM, Attia MM, Rabab MEK, Shalaby HA, Massoud AM, et al. The insecticidal activity of two medicinal plants (Commiphora molmol) and (Balanites aegyptiaca) against the blowfly Lucilia sericata (Diptera: Calliphoridae). *International Journal of Advanced Research Biological Science*. 2016;3(3):144–158. Available from: https://ijarbs.com/pdfcopy/mar2016/ijarbs18.pdf.
23) Cashman JS, Cozier GE, Harrison C, Isaac RE, Acharya KR. rystal structures of angiotensin-converting enzyme from Anopheles gambiae in its native form and with a bound inhibitor. *The Biochemical journal*;476(22):3505–3520. Available from: https://doi.org/10.1042/BCJ20190635.
24) Dallakyan S, Olson AJ. Small-molecule library screening bydocking with PyRx. *Methods in Molecular Biology*. 2015;1263:243–250. Available from: https://doi.org/10.1007/978-1-4939-2269-7_19.
25) Mahnashi MH, Alyami BA, Alqahtani YS, Jan MS, Rashid U, Sadiq A, et al. Phytochemical profiling of bioactive compounds, anti-inflammatory and analgesic potentials of Habenaria digitata Lindl.: Molecular docking based synergistic effect of the identified compounds. *Journal of ethnopharmacology*. 2021;273(113976). Available from: https://doi.org/10.1016/j.jep.2021.113976.
26) Nwakulite A, Obeagu EI, Nwanjo HU, Nwosu DC, Nnatuanya IN, Eze R, et al. Studies on Molecular Docking of Moringa Oleifera Leaf Phytochemical Constituents on Alpha Glucosidase, Alpha Amylase and Dipeptidyl Peptidase. *Journal of Pharmaceutical Research International*. 2021;p. 239–284. Available from: 10.9734/JPRI/2021/v33i28A31527.
27) Saber FR, Ashour RM, El-Halawany AM, Mahomoodally MF, Gunes AK, Zengin G, et al. Phytochemical profile, enzyme inhibition activity and molecular docking analysis of Feijoa sellowiana O. Berg. *Journal of Enzyme Inhibition and Medicinal Chemistry*. 2021;36(1):618–644. Available from: https://doi.org/10.1080/14756366.2021.1880397.