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

Abstract

**Objectives:** This study aimed to identify phytochemicals from *Momordica tuberosa* (*M. tuberosa*) leaves and its 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. tuberosa* 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; angiotensin converting 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

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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 angiotensin-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\(^11\).

## 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. 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. 

| 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), |
| 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 | 7340104 | 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 | 166240  | 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  | 2917944 | 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 |
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 AnoACE 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 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).

Fig 1. A - GC-MS spectrums interpretation

Fig 2. a, b, c - GCMS spectrum interpretation
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. 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. 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](https://www.indjst.org/)

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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.

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