Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 20 | No.3 | pp. 1237-1242 | 2021

Original Research Paper

https://doi.org/10.46488/NEPT.2021.v20i03.033

Open Access Journal

Insilico Molecular Docking Studies of Volatile Compounds Identified by GC-MS from *Tagetes* Species Against *Mamestra brassicae* (Linnaeus, 1758)

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 26-08-2020
Revised: 29-09-2020
Accepted: 15-10-2020

Key Words:
Insect Repellents
Tagetes species
GC-MS
Molecular docking
Mamestra brassicae

ABSTRACT

Plants evolved to be a potential source of pharmacologically active compounds that are being widely accepted as insect repellent compounds for generations. Products of natural origin are mostly preferred over synthetic compounds because of fewer side effects on human health and the environment, have the potential to be produced locally, cost-effective, and are proved to be more efficient. They are best suited in organic food production and can play a much greater role in developing countries as a new class of eco-friendly products for controlling pests. In turn, the development of repellents is desirable alternatives to synthetic chemical insecticides for controlling pests. In the process of continual search for insect-based repellents of natural origin, a wide number of *Tagetes* species have been archived and all parts of this plant from root to seed possess a range of phytochemicals that are responsible for the repellent activity. The present study concentrates on the identification of active volatile compounds from *Tageteserecta* leaves by Gas chromatography-mass spectrometry (GC-MS) analysis and further evaluation through molecular docking studies of identified compounds against *Mamestra brassicae*.

INTRODUCTION

Over the decades, the tradition of utilizing plant derivatives as a potential defense barrier against a wide variety of insects has been globally accepted (Luthria et al.1993). In numerous cases, the botanicals have a long history of usage as traditional remedies to kill or repel insects and are in continual use to date (Broussalis et al.1999). It was estimated that around 2000 species of botanicals are known to inherit some insecticidal activity (Klocke 1989). It is a well-known fact that solvent extracts and other secondary metabolites especially essential oils of many plants show differential levels of insect or bug-repellent properties (Chogo & Crank 1981, Curtis et al. 1991, Trigg & Hill 1996, Thorsell et al. 1998).

Tagetes that originates from the Asteraceae family is an annual, herbaceous ornamental plant that is reported native to Mexico (Tosco1970). It is a genus probably recognized as a source of natural colors (Timberlake & Henry 1986). The genus is also known to have very attractive biologically active compounds such as essential oils (Marotti et al. 1996) and thiophenes (Hulst et al. 1989) that are known for repelling insects (López et al. 2011). In the process of evolution, the interplay between plants and pests or insects is an important determinant of plant yield. These plant-pest interactions that include volatile chemical passages have been widely utilized in the regulation of agricultural pests. In response to the attack, plants have developed a scope of resistance mechanisms to lessen the risk of damage and loss of yield or productivity (Mitchell et al. 2016).

The present work involves the isolation of the volatile compounds of *Tagetes* species by using activated charcoal and analyzing those compounds by GC-MS and also describes the further evaluation of identified compounds against *Mamestra brassicae* through molecular docking studies.

MATERIALS AND METHODS

Viton-lined glass lid, glass jars, aluminum foil, pot, activated charcoal, steel cartridge, pump, N-hexane, methanol, and acetyl chloride

Collection of Plant Material

Tagetes errecta plants were collected from the fields which were maintained in the good physical condition and were grown to a height of 20 centimeters suitable for the extraction experiment.

Extraction and Purification of Volatile Compounds

Extraction of volatile compounds is carried out in a closed-loop stripping system as described by Boland et al. (1984) in which the entire plant is placed in a pot and covered with an aluminum foil and kept inside the closed glass jar which consists of a Viton-lined glass lid with an inlet and outlet.

Air is continuously circulated and the emitted volatile compounds were collected from empty glass jars and also from aluminum-wrapped pots filled with autoclaved soil to collect non-plant-related volatiles. Prior to the collection of volatiles, one end is allowed to supply air through the aquarium motor air pump and on the other side, approximately 100 mg of activated charcoal in the cotton plug is fixed. Plant volatiles were collected in a stainless steel cartridge by drawing air from the glass jars by using an external pump. The whole jar is enclosed without any air leakage either inside or outside (Kroes et al. 2017). The extracted volatile compounds from charcoal were further purified by soxhlet extraction. A sample of organic volatile compounds was extracted by n-hexane in soxhlet extraction. This sample was mixed with equal volumes of methanol and acetyl chloride to attain a final concentration of up to 10 mL.

GC-MS Analysis

The identification and quantification of plant volatiles were performed according to the method described by Pangesti et al. (2015). The plant volatiles were separated and detected using a thermo trace ultra gas chromatograph (GC) which was coupled to a thermo trace DSQ quadrupole mass spectrometer (MS) (Thermo Fisher Scientific, Waltham, USA). The volatiles were thermally discharged from the Tenax TA cartridges at 250 °C for 10 min with a helium stream of 20 mL min^{-1} on an Ultra 50:50 warm desorption unit (Markes, Llantrisant, UK). A cool sorbent trap at 0°C (Unity, Markes) was focused, and after the end of the desorption procedure, volatiles were discharged from the cold trap by ballistic warming at 40°C s^{-1} to 280°C, which was kept up for 10 min and was then moved in a splitless mode to an analytical column [(ZB-5MSi; 30 m × 0.25 mm i.d. × 0.25 µm film thickness with 5 m implicit guard column (Phenomenex, Torrance, CA, USA)] arranged inside the GC oven. The temperature of the GC oven was at first held at 40°C for 2 min, which was then maintained at 10°C min^{-1} and finally raised to a final temperature of 280°C and held for 4 min under a helium stream of 1 mL min^{-1}. The DSQ MS was worked in a scan mode with 35–350 amu mass range at 5.38 scans s^{-1} and spectra were recorded in electron impact ionization (EI) mode at 70 eV. MS transfer line and particle or ion source were set to 275°C and 250°C, separately. Volatiles were probably distinguished by correlation of mass spectra with those in the NIST 2005 and the Wageningen Mass Spectral Database of Natural Products MS libraries, as well as utilizing experimentally acquired Linear Retention Indices (LRI) (Kroes et al.2017).

Molecular Docking

Currently, multiple docking tools have been in use that run based on structure-based drug design strategies. The auto dock is one of the componential software tools that work on such a strategy. In the present work, the molecular docking studies were performed using auto dock – docking tool module to study the intermolecular interactions of chemosensory protein2 (CSP2) identified from the moth *M. brassicae* with standard lead inhibitor DEET (N,N-Diethyl-meta-toluamide) at the active site 3D space of protein of interest and also used to study the binding energy of lead inhibitor DEET.

Protein Structure Preparation

A typical protein structure was obtained from a protein data bank (PDB) with a PDB ID as 1k19 (www.pdb.org/pdb/) (Runthala A et al. 2010) for the molecular docking study; *i.e.* CSP2 structure PDB ID was 1k19. To obtain the protein structure, all hydrogen atoms were added, removed water molecules from the cavity, lower occupancy residue structures were deleted, filled the missing residues, generated the side chains and any incomplete side chains were replaced using the ADT version 4.2. The suitable ligand structures for docking were then saved in PDBQT file format.

Preparation of Ligands

ChemDraw Ultra 7.0 was utilized in the designing of the structure of the 2D light and these structures were changed over to 3D structures utilizing Chem3D ultra 7.0. MM2 was used for the minimization of energy levels. These energy-minimized ligands were utilized for docking assessment. All ligand structures were saved in .pdb file format to provide as an input to 2 (Version AutoDock 4.2). After evaluation, the ligand structures were saved in the PDBQT record design.

Validation of Molecular Docking

Protein-Ligand interactions happen through the sub-atomic mechanics resulting in the conformational changes in which the ligand-binding changes the protein state and its function. To know the reliability of such interactions, the technique of molecular docking should be validated preliminarily for the analysis of ligands. The co-crystallized ligand was extracted from the CSP2 and re-docked onto its active dynamic site. The reference standard ligand (DEET) was extracted from the PDB and re-docked again onto the active site to compute the docking energy level.

Auto Docking

AutoDock vina version (4.0) was used for molecular docking studies which utilize gradient-based conformational search. Volatile chemical compounds namely docosane, docosanoic acid, eicosane, heneicosane, heptacosane, heptadecanedipentyl, hexacosane, hexadecanoic acid methylester, hexatriacontane, octacosane, octadecanoic acid methylester,

pentacosane, pentacosane-13 undecyl, tetracosane and a standard active ingredient in most of the insect repellents i.e. DEET were docked against 1k19 and results were analyzed based on their binding energies. Based on binding free energies almost for all ligands, 100 stimulations were performed for every docking experiment. The relatedness of the docked structure was estimated by calculating the root mean square deviation (RMSD) (Runthala et al. 2010) between the coordinates of the atoms. The least binding energy conformations were considered as the best docking posture.

RESULTS AND DISCUSSION

In the context of farming, insect or pests management plays a vital role in deciding the yield of the crop. Presently, a wide range of synthetic insect repellents or insecticidal compounds is in trade. The most common of all the synthetic repellents with an excellent insect repellency property used worldwide is DEET (Fig.1) (N,N- diethyl-3-methylbenzamide) (Yap 1986, Coleman et al. 1993, Walker et al. 1996). Although it is widely accepted its usage is limited because of its noticeable effects like unpleasant odor, it has the ability to penetrate the skin, damages the plastic, painted surfaces and synthetic fabrics thus resulting in a search for new alternative approaches (Miller 1982, Roland et al. 1985, Briassoulis 2001, Clem et al. 1993). A substitute for synthetic repellents is to use natural products with good efficacy and which are eco-friendly. Botanical insecticides have been recommended as an alternative to synthetic insecticides for reducing the loss of crop yield that is being invaded by various pests or insects and pose only a negligible threat to the environment and human health. However, only a few of the botanicals are in current use in the farming community and there is an urgent need for the development and trading of new botanical insecticides (Isman 2006). The tradition of using plant-derived compounds, particularly essential oils, has been recently launched into the market which is found to be active against hematophagous arthropods (Curtis et al. 1991).

A wide variety of *Tagetes* species have gained researchers interest as it possesses essential volatiles with a remarkable insecticidal property. The essential volatile compounds of the *T. errecta* plant were extracted by using charcoal and further purified. The volatile compounds present in *T. errecta* were identified by the GC-MS technique. Molecular

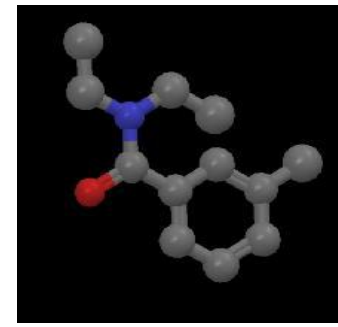


Fig. 1: Structure of DEET.

Fig. 2: Structure of 1k19.

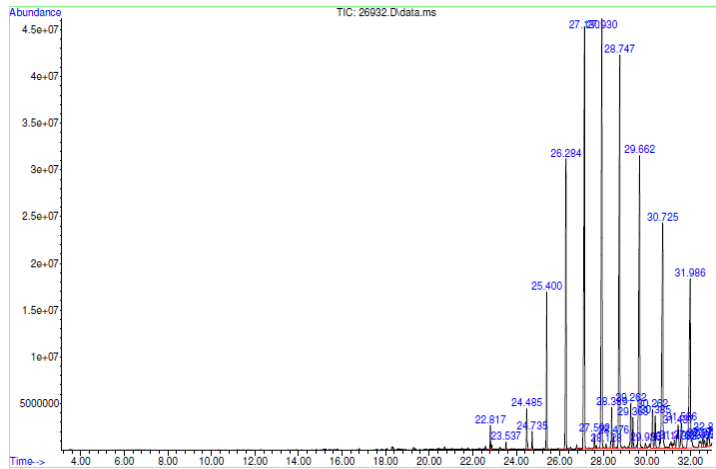
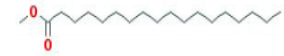
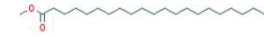


Fig. 3: GC-MS chromatogram of volatile compounds of *Tagetes errecta.*

Table 1: Volatile Compounds of *Tagetes errecta* obtained by GC MS reports.

Peak	R. T. Min	Compound name	Structure	Peak height	Corr. Area	Quality	Structure
1	22.817	Hexadecanoicacid,methyl ester	$C_{17}H_{34}O_2$	2397957	42882341	98	
2	23.537	Eicosane	$C_{20}H_{42}$	779053	14533637	98	
3	24.485	Heneicosane	$C_{21}H_{44}$	4207688	90714880	99	
4	24.735	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	1915660	33984466	99	
5	25.400	Docosane	$C_{22}H_{46}$	14962963	304217950	96	
6	27.130	Tetracosane	$C_{24}H_{50}$	42719981	1280463770	99	
7	27.592	Heptadecane, 8,8- dipentyl-	$C_{27}H_{56}$	1474031	2741475	91	
8	27.930	Pentacosane	$C_{25}H_{52}$	42116615	1311990887	94	
9	28.128	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	467389	11435367	99	
10	28.389	Hexatriacontane	$C_{36}H_{74}$	4144778	93809392	97	
11	28.476	Pentacosane,13-undecyl-	$C_{36}H_{74}$	1260881	27132915	72	
12	28.747	Hexacosane	$C_{26}H_{54}$	39741656	1255452851	98	
13	29.262	Tetracosane	$C_{24}H_{50}$	4529030	117897533	98	
14	29.363	Pentacosane	$C_{25}H_{52}$	2991924	67853632	89	
15	29.662	Heptacosane	$C_{27}H_{56}$	30665082	1053795210	98	
16	29.936	Octacosane	$C_{28}H_{58}$	481534	17865430	98	
17	31.986	Octacosane	$C_{28}H_{58}$		803393205	99	

docking studies reveal the presence of novel compounds by comparing their highest binding affinity with Chemosensory Protein CSP2 (1k19) (Fig. 2) identified from the moth *M. brassicae.* The GC-MS analysis shown in Fig.3 reveals that 14 volatile compounds were present in extracts of *T. errecta* including docosane, docosanoic acid, eicosane, heneicosane, heptacosane, heptadecanedipentyl, hexacosane, hexadecanoicacidmethylester, hexatriacontane, octacosane, pentacosane, tetracosane octadecanoic acid methylester, pentacosane13undecyl (Table 1). From the GC-MS analysis tetracosane, pentacosane, hexacosane, heptacosane are the compounds present more in the volatile organic compounds of *T. erecta.* Based on the correlation area of the peaks of GC-MS results, pentacosane is the highest amount present in the sample with a correlation area of 1311990887, the second-highest compound is tetracosane with a correlation area of 1280463770, and the least amount of compound is Heptadecane, 8,8- dipentyl- with a correlation area of 2741475.

To explore the accurate intermolecular interactions between the ligand and the protein target, molecular docking studies were performed. AutoDock vina version 4.0 was used which executes grid-based ligand docking between tiny ligand molecules and complex receptor molecules which

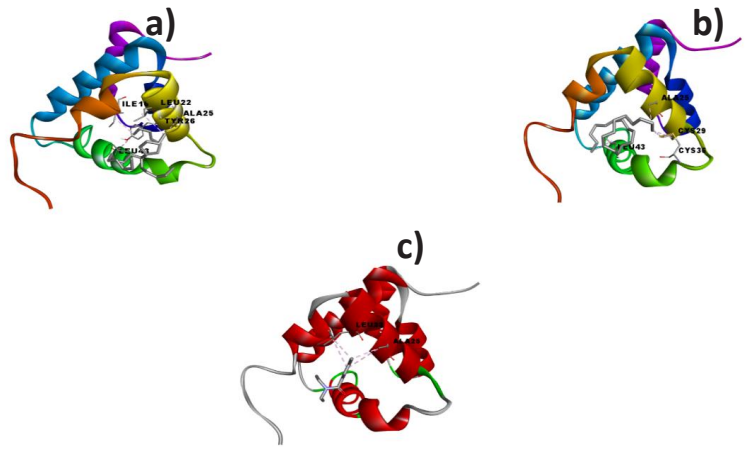


Fig. 4: Docking of a) Heptadecanediphenyl ,b) Docosane, c) DEET with 1k19.

usually is a protein of interest. The 3-dimensional structural information of the protein of interest i.e Chemosensory Protein CSP2 identified from the moth *M. brassicae* was taken from the Protein Data Bank (PDB) with PDB ID as 1k19. Initially, the protein preparation was performed by deleting the water molecules, adding the hydrogen bonds, and by generating the side chains. The volatile compounds extracted from the *T. erecta* species obtained from GC-MS analysis were docked into the active site of 1k19. Molecular docking methodologies are basically used in the current generation drug design process to figure out the protein-ligand interactions **(**Gaddaguti et al.2012**)**. Thus, understanding the detailed 3D structure of a protein-ligand composite at the very basic atomic level could be served as a significant subject in biological sciences (Gaddaguti et al. 2012, Ahmed et al. 2018). The most accurate method of determining the exactness of a docking protocol is to determine how closely the lowest energy pose i.e the binding conformation is predicted between the ligand and the target protein.

All the volatile compounds obtained from the GC-MS analysis of *T. erecta* were docked with the CSP2 protein 1k19. Among the volatile compounds (ligands) identified through GC-MS analysis docked in this study (Fig. 4), two compounds, namely, heptadecanediphenyl and docosane showed lower binding energy as -4.8 than the standard inhibitor DEET which showed the binding energy as -6.2 (Table 2). The least binding energy conformation pose was identified and this energy was regarded as a favorable docking pose (Ghosh et al. 2017). These volatile botanical compounds of *T. erecta* have potential insect repellency property activity against 1k19 Chemosensory Protein CSP2 of the moth *Mamestra brassicae*.

Table 2: Binding free energies for the insilco binding of ligands with 1k19.

S.No.	Ligand Name	Binding Free Energy (kCal.mol^{-1})
1	Heptadecanediphenyl	-4.8
2	Docosane	-4.8
3	DEET	-6.2

CONCLUSION

Plant-derived products (PDPs) are very useful on grounds with low mammalian toxicity, reduced environmental persistence, and complex chemistries that limit the development of pest resistance against them. In turn, for controlling pests, the development of repellents from PDPs is the best alternatives to chemical insecticides. In the present study, among 14 volatile botanical compounds screened with autodocking, two compounds namely heptadecanediphenyl and docosane showed lower binding energy than the standard compound DEET. These two bioactive volatile compounds of *T. erecta* have potential insect repellency property activity against 1k19 Chemosensory Protein CSP2 of the moth *M. brassicae*. The results of this study could be used for further characterization and in vivo inhibitor activity studies against moth *M. brassicae.*

LIST OF ABBREVIATIONS

GC-MS-Gas chromatography-Mass spectrometry, mg-milligram, DSQ-Dual Stage Quadrupole, °C-degree centigrade, min-minute, mL-millilitre, min−1-per minute, s−1-per second, amu-atomic mass unit, eV-Electron volt, NIST-National Institute of Standards and Technology, PDBQT- Protein Data

Bank, Partial Charge (Q) & Atom Type (T), MM2-Molecular Mechanics

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