ISSN: 2278-778X
Research Article
www.ijbio.com

GC-MS ANALYSIS AND *IN SILICO* MOLECULAR DOCKING STUDIES OF MOSQUITO REPELLENT COMPOUNDS FROM *HYPTIS SUAVEOLENS L*

Venugopal Gaddaguti, S Jwala Mounika, K Sowjanya, T Rao, MSR Krishna Chakravarthy R, Allu Prasada Rao*
Centre for Plant Tissue Culture and Breeding, Department of Biotechnology, KL University, Vaddeswaram, Guntur (District), A.P, India.

***Corresponding Author**: **Dr. Allu Prasada Rao,** Professor, Centre for Plant Tissue Culture and Breeding, Department of Biotechnology, KL University, Vaddeswaram, Guntur (District), A.P, India.

Received for publication: June 3, 2012; **Accepted**: August 25, 2012.

Abstract: Plants constitute major source of drugs for prevention and spread of wide range of pathogenic carriers and also treating various diseases of human beings. Modern people increasingly prefer drugs of natural origin mostly from plant origin due to abundant accessibility and fewer side effects. Whereas synthetic drugs and antibiotics often cause wide spread toxicity and harmful side effects to the end user other than targeted health condition / pathogen carrier. In search of novel active compounds from plant origin, and to assess the efficient thereupatic properties with minimum side effects, application of advanced methods like GC MS and computational techniques play a crucial role in designing and development of drug of interest. 13 compounds were identified in aerial parts of *Hyptis suaveolens L*. methanolic extracts. Of the 13 compounds identified in the methonolic extract, Stigmast -5-en-3-ol, oleate, and Gamma-sitosterol and Butyl 11-eicosenoate found to represent 51.7% of the 13 compounds. Molecular docking studies were performed for all 13 compounds along with commercially known mosquito repellent compounds including DEET, Prallathrin, and Permithrin against Odorant Binding Protein (3N7H) of Anopheles *gambiae* using Schrodinger Maestro software. The binding affinities for compounds of *Hyptis suaveolens* were compared with known mosquito repellents for its ability to suppress human seeking behavior of mosquitoes and further possibility for designing of potential mosquito repellent natural compounds were discussed.

Keywords: *Hyptis suaveolens*, Molecular Docking**,** Odorant Binding Protein, *Anopheles gambiae*.

INTRODUCTION

Molecular docking approaches are generally used in modern drug design process to understand the protein ligand interactions. The three-dimensional structure of the protein-ligand composite could be served as a considerable source of understanding the way of proteins interact with one another and perform biological functions. Thus, knowing the detailed structure of protein-ligand and its complexes in atomic level is one of the significant issues in biological sciences. However, in the databank of proteins where in most of the docking studies, conformational changes occur on ligand binding. This may occupy small side chain rotations to increase interactions with the ligand. Molecular Docking and Virtual Screening based studies on molecular level have become an integral part of many modern structure-based drug discovery efforts. Hence, knowledge of the protein and ligand interactions with the specific drugs may provide a significant insight into the binding interactions and relativeness of the drug.

Genetic diversity of traditional medicinal herbs and plants are vulnerable by extinction as a consequence of over exploitation, environment unfriendly harvesting techniques, loss of growth habitats and uncontrolled trade of medicinal plants. *Hyptis suaveolens* is a wild plant generally known as the "Sirna Thulasi" or "Adavi Thulasi" belongs to the family of Lamiaceae [2]. It is known as "Bush-Tea- Bush" in English. *H. suaveolens* has recently been shown to possess insecticidal properties as well as grain protectant from Cowpea weevil during storage. The plant products of *H. suveolens* shows effect on the survival and reproductive potential of *T. granarium* is required for proper preservation of the groundnut seeds [3][4]**.** The extracts of *H.suveolens* play a significant role in pre-harvest maize protection against stem borers. Essential oils of *H. suaveolens* known to exhibit antioxidant activity and antimicrobial activities**.** Studies also reveal that the aqueous extracts leaves exhibit acetaminophen induced hepatoprotective activity [5]. Leaves and twigs are considered to be antispasmodic and used in anti rheumatic and anti soporific baths**.** The extracts of *H. suaveolens* are the source of natural insecticides for mosquito control**.** *Hyptis* is also used as an appetizing agent, to combat indigestion, stomach pain, nausea and infection of the gall bladder [6].

MATERIALS AND METHODS

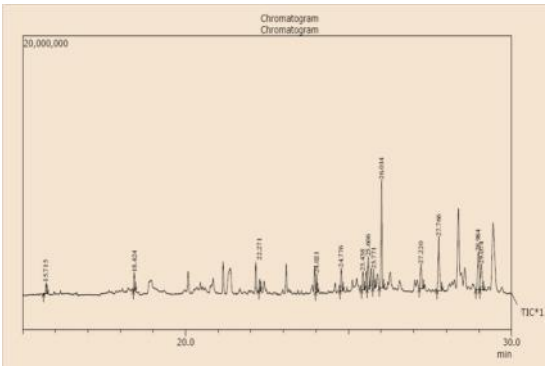
The aerial parts of *H. suveolens* plant material were collected from kondapalli reserve forest lies between Latitude.16.36°N, Longitude. 80.30°E at height about 168 meters above MSL in the Krishna district, Andhra Pradesh, India. This plant was identified with the help of regional floras [7] and taxonomists and finally confirmed with the herbarium of Botanical Survey of India.

Extraction of plant samples: Fresh leaves were sterilized, shade dried and powdered in a blender to get fine powder. 100gm of leaf powder is mixed with 1000ml (1:10 ratio) of methanol in a Schott Duran bottle and kept as air tight for 48 hours on magnetic stirrer with continuous stirring for proper mixing of powdered samples. The solutions were subjected to distillation and the extracts were subjected to GC/MS studies.

Gas chromatography-mass spectrometry analysis: The phyto chemicals were analyzed by GC-MS (SHIMADZU QP 2010) employing the electron impact (EI) mode (ionizing potential 70eV) and a capillary column (Resteck-624 ms) (30 m × 0.32 mm, film thickness 1.8µm) packed with 5% phenyl dimethyl silicone) and the ion source temperature was monitored at 200 °C. Further, the GC/MS settings were as follows: the initial column temperature was set at 45°C and held hold for 4 min; the temperature was increased to 50°C and then increased to 175°C at a rate of 10°C/min for 2min, and then finally programmed to 240°C at a rate of 25°C/min, and kept isothermal for 2min. The column oven temperature was maintained at 280°C. Helium was used as carrier gas with 99.9995% purity. Flow rate 1.491mL/min. Split ratio, 1:10.

Identification of components: The fraction composition of the samples was computed from the GC peak areas. Library searches were approved out using the WILEY8, NIST08s and FAME Library.

Figure.1: GC-MS chromatogram of *Hyptis suaveolens* methanolic extract



Gas chromatography and mass spectrophotometer analysis: According to the works of Abagli et al, [8] on chemical mosquito repellent DEET for personal protection against mosquito bites by using the natural essential oils of bush mint, and *H. suaveolens* concludes that there is no significant difference between 10% *H. suaveolens* essential oil and DEET indicating that both products are similarly effective.

Molecular Docking is the process in which two molecules fit together in 3D space. It is a key tool in structural biology and computer-aided drug design [9]. In this study, the structures were drawn by using ISIS/Draw[10], a chemical structure drawing program for Windows. By using Tsar's easy-to-use chemical spreadsheet interface the limits was observed and converted 2D structures to 3D with physicochemical properties to analyze and promote activity. For the molecular docking analysis the Schrodinger aided drug design software [11] was used.

According to Bhattacharjee [12], molecular similarity analysis of stereo-electronic properties between natural insect juvenile hormone (JH), -a synthetic insect juvenile hormone mimic (JH-mimic, undecen-2-yl carbamate), and N, N-diethyl-m-toluamide (DEET) and its analogs reveals similarities which may aid the design of more efficacious insect repellents and give a better insight into the mechanism of repellent action [13][14]. Quantitative structure Similarity analyses of stereo-electronic properties such as structural parameters, atomic charges, dipole moments, molecular electrostatic potentials and highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies were performed on JH, JH-mimic and the DEET compounds. By using compounds from *Hyptis suaveolens* obtained by GC MS reports were analyzed by molecular docking analysis.

Steps in Molecular Docking by Schrodinger:

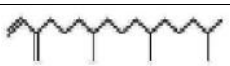
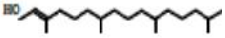
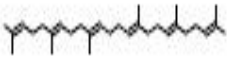
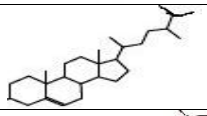
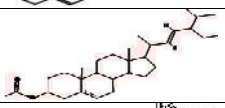
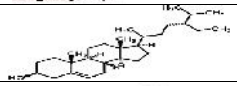
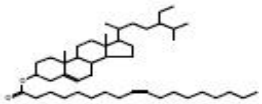
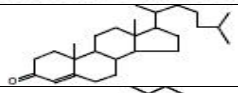
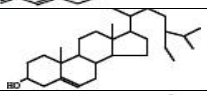
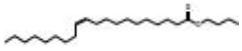
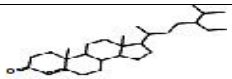
1. Building the Receptor: The 3D structure of the receptor has been downloaded from PDB and modified [15]. This included removal of the water molecules from the cavity, stabilizing charges, filling in the missing residues, generation the side chains etc according to the parameters available. After modification of the receptor it is biologically active and stable.

2. Identification of the active site: The receptor was built; the active site within the receptor was identified. The receptor may have many active sites but the one of the active site was selected. Most of the water molecules and hetero atoms presented were removed [16].

3. Ligand Preparation: Ligands can be obtained from various databases like ZINC; Pubchem can be sketched using tools like Chemsketch [17]. While selecting the ligands, the LIPINSKY'S RULE OF 5 was applied. The rule is important for drug development where a pharmacologically active lead structure is optimized stepwise for increased activity and selectivity, as well as drug-like properties, Ligand prepared according to the of lipinsky's rule (not more than 5 –H bond donors, Molecular Weight not more than 500 Daltons, Log P not over 5 for octanol water partition coefficient, not more than 10 H bond acceptors).

4. Docking: The ligand is docked onto the receptor and the interactions were checked. The scoring function generates scores depending on which the ligand with the best fit was selected. Docked compounds screened against the 3N7H Odorant Binding Protein 1 of *Anopheles gambiae* [18] with its Glide docking scores of DEET, and gamma sitosterol of *H. suaveolens* with AgamOBP1 [19] were recorded and discussed the results.

Table 1: Compounds of *Hyptis suaveolens* obtained by GC MS reports

PEAK	R.TIME	AREA (%)	NAME OF THE COMPOUND	STRUCTURE
1	15.715	1.58	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE	
2	18.424	3.17	Phytol	
3	22.271	2.07	Sylvenone	Structure not available
4	24.021	3.15	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23 -hexamethyl-, (all-E)-	
5	24.776	4.62	Tetracosamethyl-cyclododecasiloxane	
6	25.438	4.46	ERGOST-5-EN-3-OL, (3.BETA.)-	
7	25.606	9.10	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.,22Z)-	
8	25.771	3.55	BETA-DIHYDROFUCOSTEROL	
9	26.014	20.89	Stigmast-5-en-3-ol, oleate	
10	27.220	7.20	Cholest-4-en-3-one	
11	27.766	16.61	gamma.-Sitosterol	
12	28.948	14.12	Butyl 11-eicosenoate	
13	29.074	9.46	DELTA.4-SITOSTEROL-3-ONE	

RESULTS AND DISCUSSION

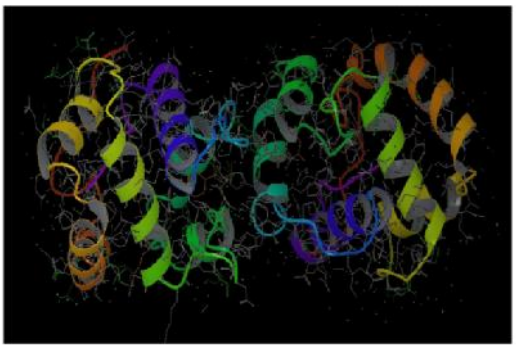
Stigmast -5-en-3-ol, oleate shows highest peak at retention time of 26.014 with 20.89% of area followed by gamma-sitosterol with peak retention time at 27.766 with 16.61% of area and butyl 11-eicosenoate with retention of 28.984 with 14.12 % of area. Among all methonolic compounds of *H. suaveolens* tested in the present study, gamma sitosterol exhibit insect maximum repellent activity when compared with known DEET against 3N7H Crystal structure of dorant Binding Protein 1 from *Anopheles gambiae*. The inter-activeness of the compounds with the amino acid residues of the 3N7H protein at the active site region was confirmed using Schrodinger Computer Aided drug Design Software. The results clearly established high binding affinity of the gamma sitosterol which is isolated from the methonolic extracts of *H. suaveolens* with the known predominant odor binding protein compounds including Decanol. Results are tabulated in table 2. Amongst the methonolic extracts of thirteen compounds of *H. suaveolens* studied in the present docking study. DEET, Gamma sitosterol, and butyl with 3N7H protein showing the interaction of amino acid residues and the hydrophobic binding pocket surrounding DEET and gamma-sitosterol confirming the affinity was reconfirmed. The docking scores for the DEET is -6.02 without hydrogen bonds as depicted in Table 2 and the docking scores of gamma sitosterol is -5.99 with hydrogen bonding, and is significantly equal to the DEET. Butyl shows very low binding affinity.

The similarity of stereo-electronic attributes of the amide or ester moiety, the negative electrostatic potential regions beyond the Vander Waals surface, and the large distribution of hydrophobic regions in the compounds appear to be the three important factors leading to a similar interaction with the JH receptor. The similarity of electrostatic profiles beyond the Vander Waals surface is likely to play a crucial role in molecular recognition interaction with the JH receptor from a distance and suggested that electrostatic bio isosterism of the amide group of the DEET compounds and JH-mimic and, thus, a model for molecular recognition at the JH receptor and the insect repellent property of the DEET analogs may be attributed to a conflict of complementarities for the JH receptor binding sites.

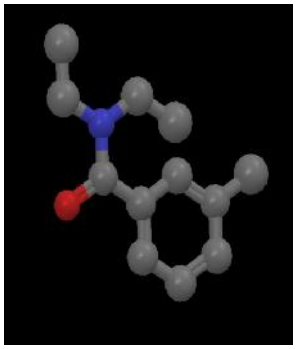
Table 2: Glide scores for the Insilco binding of Ligands with Receptor 3N7H

S.No	Ligand	G-Score	H-Bond	Residue
1	DEET	-6.0	-	-
2	GAMMA.-SITOSTEROL	-5.99	0.2	Gly 92

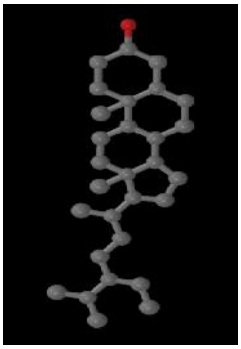
3N7H Protein



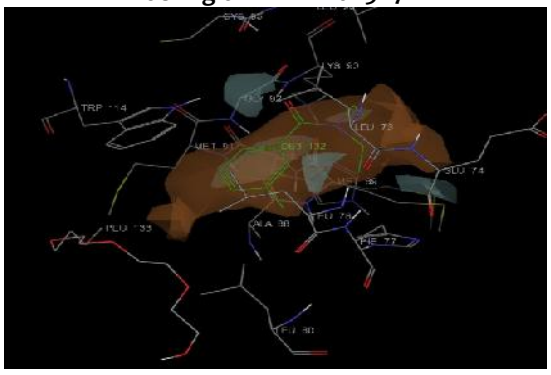
DEET



Gamma Sitosterol



Docking of DEET with 3N7H



Docking of Gamma Sitosterol with 3N7H

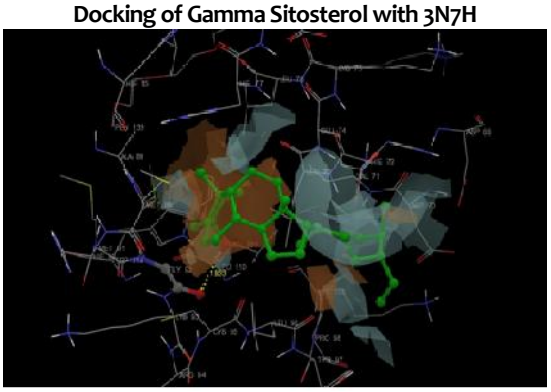


Figure 2: Docking of 3N7H Odorant binding protein crystal Structure with DEET and Gamma Sitosterol ligands.

Glide docking scores of DEET, and gamma sitosterol of *H. suaveolens* with AgamOBP1 of *Anopheles gambiae* and amino acid residues of OBP Interacting with the corresponding compounds as discussed here. DEET a synthetic mosquito repellent is considered as reference ligand for the docking study. All compounds with major area % obtained from the GC-MS analysis of *H. suaveolens* are docked with the odorant binding protein 3N7H. The docking results are shown in the table 2. The G-score, H-Bond and residue interaction shows binding affinity of the Ligands towards protein 3N7H. The G-Score of DEET is -6.0, when compared with the compounds of *H. suaveolens* G-Score of Gamma-Sitosterol is -5.99, is significantly equal to DEET. The hydrogen bond energy of DEET is 0, but the hydrogen bond energy of Gamma-Sitosterol is 0.2 which is greater when compared to DEET. The increase in the number of hydrogen bonds also increases the bond energy. Commercial products in the form of creams, lotions, liquids and mats are extensively used in the recent past and the extent of expenditure on health related products for avoiding disease carriers alarmingly increased. Moreover, the synthetic chemicals which are in use provide inadequate protection and cause certain health related complications including allergy and respiratory problems. Although commercial insect repellents like DEET, Allethrin, Prallethrin and Permethrin are found to be promising in repelling insects, still there is an ample scope to discover and design novel insect repellents using appropriate technology to improve human health and thereby raise the economic status of common man.

CONCLUSION

Methanolic extracts of *Hyptis suaveolens L.* were characterized by GC-MS method and 13 compounds were docked using Schrodinger Maestro software. Among the 13 compounds, gamma sitosterol was found as an effective mosquito repellent with a Glide score equal to -5.99 at par with the DEET which has Glide score of -6.0. Since the identified compound gamma sitosterol is a natural compound with repellent activity, the compound may have better option to design efficient mosquito repellent than existing synthetic mosquito repellents such as DEET and other known mosquito repellents viz. Prallathrin, Permithrin etc. So we imply that in future this compound may be used as a mosquito repellent to prevent the mosquito-borne disease**.** In addition, combination of more than two active natural compounds of plant origin may be effective strategy to target specific odorant binding proteins of pathogenic carriers especially mosquitoes, play a crucial role for designing effective mosquito repellent could make malaria the next modern medical and public health success story.

ACKNOWLEDGEMENT

Authors grateful to the Department of Biotechnology, KL University for providing the funding and lab facilities for successful completion of the present work.

REFERENCES

1. Brooijmans, N. Kuntz, I.D, Molecular recognition and docking algorithms. Annu. Rev. Biophys. Biomol. Struct, 2003, 32, 335-373.

2. R Ashok Joseph Vijay, V Pandiyarajan, Petchimuthu K, Comparison Of Chemical Composition of the Essential Oil of Hyptis suaveolens (L.) Poit Leaves from Different Regions of Tamil Nadu. IJPSR, 2(11) 2822-2824

3. Musa AK, MC Dike, CI Amatobi, I Onu, Evaluation of methanolic extracts of selected of Selected indigenous plants material in suppressing Trogoderma everts (coleoptera: dermesidae) infestation on stored groundnuts. Journal of Agricultural Research and Policies, 2(3): 91-95.

4. AK Musa, MC Dike, Evaluation of Nitta (Hyptis suaveolens Poit.) Seed and Leaf Extracts and Seed Powder for the Control of Trogoderma granarium Everts (Coleoptera: Dermestidae) in Stored Groundnut. Onu American-Eurasian Journal of Agronomy 2 (3): 176-179.

5. Babalola OO, Ojo OE, Oloyede FA, Hepatoprotective activity of aqueous extract of the leaves of Hyptis suaveolens (l.) Poit on acetaminophen Induced hepato toxicity in rabbits. Research Journal of Chemical Sciences, Vol. 1(7), 85-88, Oct. (2011).

6. Singh HB, AK, Handique, Antifungal activity of the essential oil of Hyptis suaveolens and its efficacy in bio control measures in combination with Trichoderma harzianum. 2007, 9, 683-687

7. Oommachan M, The flora of bhopal (angiosperms); Chapter 8: Systematic Enumeration Of Species; Ed.I.Bhopal; J. K. Jain Bros. 1976;311-312

8. AZ Abagli, TBC Alavo, Essential Oil from Bush Mint, Hyptis suaveolens is as Effective as DEET for Personal Protection against Mosquito Bites. The Open Entomology Journal, 5: 45-48.

9. John Dogulas Palleti etal, Virtual Screening and Molecular Docking Analysis of Zap-70 Kinase Inhibitors, International Journal of Chemical and Analytical Science, 2011, 2(9), 1208-1211.

10. ISIS Draw 2.3, MDL Information Systems Inc. CA, USA, 2000. http://www.schrodinger.com.

11. Bhattacharjee AK, Gupta RK, Ma D, Karle JM, Molecular similarity analysis between insect juvenile hormone and N, N-diethyl-m-toluamide (DEET) analogs may aid design of novel insect repellents , 2000, J Mol Recognit. 13(4)213-20.

12. Fradin MS, Day FJ, Comparative efficacy of insect repellents against mosquito bites. 2002, N Engl Journal of Med 347 (1): 13-8.

13. Sudakin DL, Trevathan WR 2003, DEET: a review and update of safety and risk in the general population. 2003, J Toxicol Clin Toxicol 41: 831-9. www.rcsb.org/pdb:structure summary.

14. SJ Daharwal, Computer aided drug design and bioinformatics: A current tool for designing Pharmainfo.ne.

15. Li Z, Wan H, Shi Y, Ouyang P, Personal Experience with Four Kinds of Chemical Structure Drawing Software: Review on ChemDraw, ChemWindow, ISIS/Draw, and Chemsketch. 2004, J. Chem. Inf. Comput. Sci. 44 (5): 1886–1890.

16. Seyoum A, Kabiru EW, Lwande W, Killeen GF, Hassanali A, Knols BG.2002, Repellency of live potted plants against Anopheles gambiae from human baits in semi-field experimental huts.2000, Am J Trop Med Hyg 67 (2): 191-5.

17. Weigel S, Kuhlmann J, Huhnerfuss H, Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. 2002, Sci Total Environ 295: 131-41.

Source of support: Department of Biotechnology**,** KL University,
Conflict of interest: None Declared