ZEUGMA BIOLOGICAL SCIENCE
ZEUGMA BIOLOGICAL SCIENCE
2024
v:5 n:1 p:9-20

Species Variation Of Mosquito Repellent Activities From Two Medicinal Plants Extract

Odunlade A.K[1]., Faremi O.E.[2], Ajayi O.R.[3], Aina O. S.[4], Okorafor U[5] and Hameed I.O[6]
[1,2,3,5&6]Department of Biological Science, Yaba College of Technology Yaba, Lagos.
[1,4]Department of Biological Science, Trinity University, Yaba, Lagos.

akodunlade@gmail.com

ABSTRACT

The campaign for living in a friendly environment by reducing the rate of pollution through the use of biodegradable substance most especially plant materials for the control of mosquito is major priority of a, nation. Over the years, mosquito have been controlled by the use of synthetic chemical compounds and this is known to left behind toxic chemical residue which get accumulated into the food chain. This study was carried out to evaluate the efficacies of the crude extract of the peel of lemon *(Citrus sinensis)* and *Azadirachta indica* using separating funnels. The mosquito' repellency of the extract from the two plants were evaluated using the human bait technique where by hand treated with different concentration of extract from 10mg/ml, 20mg/mL 30mg/ml, 40mg/ml. 50mg/ml. Petroleum ether extract of lemon peels showed higher larvicidal and repellent activity thaTn ethanol extract for each of the plant. The value of the lethal concentration causing 50% mortality (LC_{50}) of petroleum ether of lemon peel (LC_{50} =34.52), while *Azadirachta indica* (LC_{50} 98.72). The ethanol extract of lemon peel (LC_{50}=80.32) while that of *Azadirachta indica* (LC_{50} = 44.32). The plant extract tested showed variable effect on pupation and adult emergence and different morphogenic abnormalities were observed in immature and adult stages. All concentration of plant extracts used in the present study exhibited a repellent activity against adults with effectiveness depending on the solvent for extraction. The most effective plant extract exhibiting 100% repellency or biting deterrence against *Anopheles gambiae* was the petroleum ether extract of lemon peel at all dosage. Result of the study may contribute to design an alternative way to control mosquitoes currently base on applications of synthetic insecticides. These extracts could be developed commercially as an effective personal protection measure against mosquito bites and to control diseases caused by mosquito-borne pathogen. In-silico studies also revealed that the main components of theses extracts are potential antimalarial inhibitors (Terpene: -6.6 kcal/mol) with comparable binding energy with Azadirachtin (-7.5 kcal/mol) and DEET (-6.5 kcal/mol) references binding with histo-aspartic protease (pdb id: 3qvc) of *Plasmodium falciparum.*

Keywords: mosquito repellent, *Citrus cinensis, Azadirachta indica,* histo-aspartic protease*, Plasmodium falciparum.*

INTRODUCTION

Mosquito is the primary host in the spread of malaria. Mosquito repellents are substances that are designed to make surface unpleasant or unattractive to mosquito. They typically contain one active ingredient which aid in delivery of cosmetic appeal (WHO, 2006). Traditionally, various types of substances have been used to repel mosquitoes and these include smokes, plant extracts, tars and citronella oil, (MIM, 2004). *Azadirachta indica*, (Neem), *Jatropha curcas*,

https://doi.org/10.55549/zbs.1417938

Citrus Limon, *Moringa oleifera* are plant materials that are traditionally used and speculated to have mosquito repelling potentials (Coker *et al.*, 2000). However, certain characteristics such as volatility, limit their effectiveness. The oil components are responsible for the plants mosquito repelling activity. For a material to be valuable as a mosquito repellent, it must effectively discourage insect attack on the treated area for many hours and on many different types of surfaces, it must work in different environmental conductions, it must be environmental friendly when applied to human or animal skin, it must be cosmetically acceptable having a pleasant odour, taste and feel, it should also be harmless to clothing, it could have a relatively low cost and be effective against other common types of insects, such as flies (Oshaghi *et al*., 2003; WHO, 2008).

The high level of threat malaria poses to humanity, its increased level of mortality and morbidity rate as index of its threat; has made it to become very necessary to look further for an alternative measure for malaria prevention or control. Mosquito as a primary host to the spread of malaria is an important target and its control can improve the status of malaria free environment. Thus, there is need to evaluate plant-based repellents in order to supplement conventional control methods. Mosquito repellent properties of plants were well known before the advent of synthetic chemicals. A wide range of plants have been used for centuries for repelling mosquitoes and other insects using varying techniques such as burning plant materials to generate smoke that repels mosquitoes, hanging bruised fresh plants in houses, and placing potted plants inside houses. Studies have shown (Sofowora, 1995) that the four plant *Azadirachta indica*, (Neem), *Jatropha curcas*, *Citrus Limon*, *Moringa oleifera* extracts and their oils can serve as anti-anxiety and anti-depressant due to their active ingredients. This study is therefore designed to screen and compare their mosquito repellent activities.

METHODOLOGY

Plant collection: The plants wastes material *Citrus limon*(lemon peel) was collected from mile 12, area in Kosofe Local government of Lagos State market while *Azadirachta indical* (Neem), *Jatropha curcas*, *Moringa oleifera* was collected from Yaba College of Technology Staff Quarters, Yaba, Lagos.

Insect collection

The mosquitoes were obtained from the Insectary of the Nigerian Institute of Medical Research (NIMR). They were maintained in a white plastic bowl containing water and were fed with mice cubes. After three days the pupae metamorphosed to Adult Mosquito and these were trapped within the cage and kept at room temperature. The adult male was fed with the 10% glucose solution while the females were given blood meal from de-mobilized chicken. After fews days, eggs were collected and filter papers which were used to line the inside of the beakers and kept dried until a new generation of larvae were needed. The procedure was regularly repeated to provide larvae for Bioassay in accordance with Grieve *et al* (2012)

Preparation of solvent extract

Two hundred gram (200g) from each of the experimental plants (lemon peel, neem leaves, *Jatropha curcas*, *Moringa oleifera*) sundry and grounded to powder and were prepared separately using petroleum ether and ethanol as a solvent in accordance to (Berger and Bernett, 2013). The extraction period for each solvent was 72hrs. The solid residue of each extract was used for preparation of graded concentration in mg/ml.

https://doi.org/10.55549/zbs.1417938

Larvacidal bioassay

Larvicidal Bioassay protocol was followed in accordance to WHO (1997). The tested concentration ranged from 10mg/ml, 20mg/ml. 30mg/ml, 40mg/ml. 50mg/ml. The Larvae of the Anopheles Gambiae were used. The colony of the larvae was maintained at 28 + 2°C and 80% of Relative Humidity. The larvacidal activity was measured by placing ten larvae in a tested concentration in mg/ml of lemon peel, Neem leaves, *Jatropha curcas*, *Moringa oleifera* extracts. Each of treatment was replicated three times. Distilled water was used as control.

Larvae were taken to be dead when all bodily movement ceased and the animal cannot progress from one point to another. Mortality was assessed for 1hour, 6hours and 24hours respectively.

The dead larvae were counted and recorded. The LC_{50} values were calculated using the prohibit analysis software (SPSS).

Acute Toxicity Studies

The back hairs of Albino rats were shaved using a shaving stick to expose a skin area of about 5cm. The Albino rats were in five groups according to the tested concentrations. The exposed area of the Albino rats treated with the extract of lemon peel and neem were covered with a gauge pad (bandage). After 24hrs the tape and gauge were removed and the treated areas were evaluated for erythernatous lesions. (Redness of the skin produced by congestion of the capillaries) and endematous lesions (accumulation of excess fluid in skin and tissues). John *et al* (2005).

Repellency assay test (hand in cage method)

The mosquito repellency of the extract from lemon peel (*Citrus limon),* neem *Azadirachta indica, Jatropha curcas*, *Moringa oleifera* were evaluated by using an arm-in-cage test as described by Schreck and McGovern (1989) and WHO (1995). The technique involves counting of the number of mosquitoes biting a volunteer's hands introduced into a 14 x 14 x 17 in mosquito cage containing 50-60 of 3-5days old of male and female *Anopheles gambiae.* Counting was done for the first three minutes of every half-hour exposure.

Toxicity Prediction of lead and reference compounds

Lead and reference compounds underwent toxicity testing by inputting their SMILES representation, which was drawn using ChemDraw 14.0 and saved as an .sdf file. Protox II web server (https://tox-new.charite.de/protox_II/) was utilized for this purpose. The web server provided data on hepatoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity, which were then extracted (Banerjee *et al*., 2018). This screening aimed to investigate their toxicity profiles and assess their compliance with all drug-likeness rules as outlined by Lipinski *et al*. (2012).

Selection of Histo-Aspartic Protease (HAP) protein receptor

Crystal structure of the Histo-Aspartic Protease protein molecule, with a resolution of 2.10 Å, was acquired from the Protein Data Bank located at rcsb.org (https://www.rcsb.org/). The structure was obtained in the pdb format and subsequently processed using BIOVIA DS 2020 to remove unwanted ligands and water molecules. Additionally, polar hydrogen atoms were added to the structure as required.

https://doi.org/10.55549/zbs.1417938

In-silico drug-likeness and ADME predictions

Selected compounds also underwent drug-likeness analysis utilizing admetSAR2 (http://lmmd.ecust.edu.cn/admetsar2) to predict crucial adsorption, distribution, metabolism, and excretion (ADME) parameters for potential drug candidates (Yang *et al*., 2018). The SMILES representations of these compounds were uploaded onto the web server, and the generated results were extracted and thoroughly analyzed.

2.6. Bioactivity score

To assess their suitability as drug candidates, lead compounds and reference underwent drug-likeness analysis using admetSAR2 (http://lmmd.ecust.edu.cn/admetsar2). This analysis aimed to predict vital parameters related to adsorption, distribution, metabolism, and excretion (ADME) for these compounds, with the goal of identifying potential candidates for further drug development (Yang *et al*., 2018). The SMILES representations of the compounds were submitted to the web server, and the resulting data were carefully extracted and comprehensively examined.

2.7 Molecular docking study

To evaluate the inhibitory potential of the lead compounds, docking simulations were performed against histo-aspartic protease (HAP) using the PyRx 0.8 AutoDock Vina Wizard. The macromolecules were converted to Autodock format, and a flexible ligand to rigid protein approach was employed. All possible binding sites on the target protein were explored during the docking process. The docking calculations were performed within a cubic grid of dimensions 90 × 75 × 60 centered on the protein, encompassing the entire protein structure. This process lasted approximately one hour. A grid spacing of 1.00 Å was utilized to generate the grid maps while each ligand underwent nine independent runs to ensure accuracy. Based on the identified potential binding sites, energetically favorable binding conformations were selected using AutodockVina (Trott and Olson, 2010). The binding modes, along with their respective binding affinities and RSB (upper and lower) values, were obtained to guide the selection of the highest scoring binding conformation for each ligand. The binding mode with the best binding affinity was chosen. The ligand-protein complexes were analyzed using DS Visualizer. All software applications were executed on PC-based machines running the Microsoft Windows 10 operating system

RESULTS AND DISCUSSION

The results of this investigation showed that lemon peel *(Citrus limon)* in Petroleum Ether is more effective than Ethanol extract in all the tested concentration as shown in Table (I-II). However, Neem leaves extract in petroleum Ether is less effective when compared to the mortality mean and the result is agreement with Richard *et al* (2009); who reported the effectiveness of Mosquito Repellent Activity and Phytochemical characterization of *Ocimum bacillicum* of extract in petroleum ether. Phytochemicals are the principal active components that are believed to exhibit the medicinal activity of the plants and the repellents activity of the oils. Terpenoids are said to be responsible for the flavor of fruits, the fragrance of the flowers and the quality of agricultural products (Banthorpe, 1991; Heinrich *et al,* 2005). The presence of terpenes is speculated to be associated with fragrance material and repellent activity of oils (Coker *et al.,* 2000).

https://doi.org/10.55549/zbs.1417938

Table 1: Percentage of mortality of lemon peel *(Citrus limon)* and neem *(Azadirachta indica)* Extract on mosquito larva of *Anopheles gambiae*

	Lemon peel		**Neem leave**	
Cone, of the extract in mg/ml	**Petroleum ether mortality (%)**	**Ethanol mortality (%)**	**Petroleum ether mortality (%)**	**Ethanol mortality (%)**
10	52.4 -1-0.25	12,3+0.11	23.3+0.15	45.36+0.10
20	60.1+0.15	15.1+0.13	25.4+0.21	52.31+0.35
30	62.3 +0.27	20.0+0.21	26.3+0.35	60.11+0.22
40	60.7 +0.35	24.2+0.12	28.0+0.22	63.95+0.15
50	66.7+0.13	28.3+0.18	36.0+0.13	68.21+0.34
Control water extract	8.50+0.15	8.50+0.15	8.50±0.15	8.50+0.15

Table 2: Percentage mean repeliency of lemon peel (*Citrus limon)* and neem leaf (*Azadirachta indica*) on adult anopheles gambiae

Cone, of the extract in mg/ml	**Lemon peel**			**Neem leave**		
	Mean mortality	**Petroleum ether mortality**	**Ethanol mortality**	**Petroleum ether mortality**	**Ethanol mortality**	**Mean mortality**
10	3.05 +0.02	60.70	21.50	1.09 + 0.32	55.50	0.56 ±0.32
20	3.25 ±0.01	66.83	26.05	1.72+0.42	60.12	0.58 + 0.11
30	3.79+0.31	75.75	36.7	2.5 ±6.25	66.45	0.67 ± 0.30
40	3.80 ±0.25	78.21	40.4	2.32 +0.78	70.45	0.82±0. 0.45
50	4.56 ±0.30	86.73	42.7	2.54 ±0.41	75.79	1.36 ±0.36
Control water extract	0.67 ±0.31	8.05	8.05	0.67	8.05	0.67 ;

Each test was repeated three times for each concentration. Five different human volunteers were used per test after obtaining their consent to participate in the experiment. The repellency test was carried against male and female *Anopheles gambiae*. Control readings were obtained by placing hand inside the repellent chamber without applying any repellent extract from the tested materials.

Table 3: Analysis of the result of percentage mortality on concentration of the different plant extracts on mosquito larva showing their LC50

Average concentrations in mg/ml	**LC_{50}**	**SLOPE + S.E**	**D.F**	**PROB1T LINE EQUATION**
Lemon peel in (petroleum ether)	98.72	9.37+0.39	2	Y = 7.6322+ 9.370x
Lemon peel in (Ethanol solvent)	44.52	4.065+0.30	1	Y = 12.026+15.415X
Neem leave in (petroleum ether solvent)	34.52	2.095+0.11	1	Y- 3.963 + 7.120X
Neem leave in ethanol solvent	80.32	7.285+0.56	1	Y = 4.669
Water extract	2.34	0.02+		Y=0.15

https://doi.org/10.55549/zbs.1417938

Table 4: Percentage of mortality of lemon peel *(Citrus limon)* and neem *(Azadirachta indica)* Extract on mosquito larva of *Anopheles gambiae*

	Lemon peel		Neem leave	
Cone, of the extract in mg/ml	**Petroleum ether mortality (%)**	**Ethanol mortality (%)**	**Petroleum ether mortality (%)**	**Ethanol mortality (%)**
10	52.4 -1-0.25	12,3+0.11	23.3+0.15	45.36+0.10
20	60.1+0.15	15.1+0.13	25.4+0.21	52.31+0.35
30	62.3 +0.27	20.0+0.21	26.3+0.35	60.11+0.22
40	60.7 +0.35	24.2+0.12	28.0+0.22	63.95+0.15
50	66.7+0.13	28.3+0.18	36.0+0.13	68.21+0.34
Control water extract	8.50+0.15	8.50+0.15	8.50±0.15	8.50+0.15

Table 5: Percentage mean repeliency of lemon peel (*Citrus limon)* and neem leaf (*Azadirachta indica*) on adult anopheles gambiae

	Lemon peel			Neem leave		
Cone, of the extract in mg/ml	**Mean mortality**	**Petroleum ether mortality**	**Ethanol mortality**	**Petroleum ether mortality**	**Ethanol mortality**	**Mean mortality**
10	3.05 +0.02	60.70	21.50	1.09 + 0.32	55.50	0.56 ±0.32
20	3.25 ±0.01	66.83	26.05	1.72+0.42	60.12	0.58 + 0.11
30	3.79+0.31	75.75	36.7	2.5 ±6.25	66.45	0.67 ± 0.30
40	3.80 ±0.25	78.21	40.4	2.32 +0.78	70.45	0.82±0._0.45
50	4.56 ±0.30	86.73	42.7	2.54 ±0.41	75.79	1.36 ±0.36
Control water extract	0.67 ±0.31	8.05	8.05	0.67	8.05	0.67 ;

Richard *et al* (2008) reported that plants have different physic-chemical properties and the choice of solvent and extraction procedures is very important in other to maximize the bio-activities of the plant extract. Repellents are used as personal protection methods against mosquitoes with the major aim of avoiding nuisance (Trigg, 1996). Insect repellents are considered useful alternative where other control measures are neither practical nor possible.

Table 6: Analysis of the result of percentage mortality on concentration of the different plant extracts on mosquito larva showing their LC50

Average concentrations in mg/ml	**LC_{50}**	**SLOPE + S.E**	**D.F**	**PROB1T LINE EQUATION**
Lemon peel in (petroleum ether)	98.72	9.37+0.39	2	Y = 7.6322+ 9.370x
Lemon peel in (Ethanol solvent)	44.52	4.065+0.30	1	Y = 12.026+15.415X
Neem leave in (petroleum ether solvent)	34.52	2.095+0.11	1	Y- 3.963 + 7.120X
Neem leave in ethanol solvent	80.32	7.285+0.56	1	Y = 4.669
Water extract	2.34	0.02+		Y=0.15

https://doi.org/10.55549/zbs.1417938

Molecular modelling of Lemon peel (Citrus sinensis) and Neem leaf (Azadirachta indica)

The structures of main components of lemon peel (Terpinene) and neem leaf (Azadirachtin) are presented in Figure 1 alongside a common ingredient in mosquito repellants (DEET). The physicochemical parameters of these compounds are also presented in Table 7.

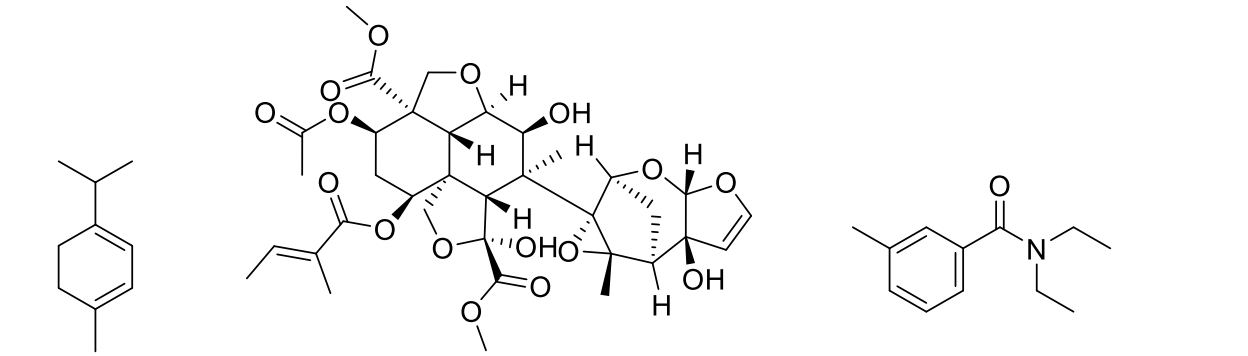

Figure 1: Structures of the prominent component of lemon peel (Terpinene), neem leaf (Azadirachtin) and a common ingredient in mosquito repellants (DEET).

Table 7: Physicochemical parameters of Terpinene, Azadirachtin and repellant reference DEET (obtained from molinspiration webserver).

Physicochemical parameters	**Terpinene (Lemon peel)**	**Azadirachtin (Neem leaf)**	**DEET (Reference repellant)**
miLogP	3.36	1.42	2.08
TPSA	0	215.37	20.31
natoms	10	51	14
MW	136.24	720.72	191.27
nON	0	16	2
nOHNH	0	3	0
nviolations	0	2	0
nrotb	1	10	3
volume	156.74	611.69	199.1

Toxicity predictions of lead components and reference

Coputational toxicity prediction/ probability model of targets such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity parameters revealed the lemon peel component, Terpene to be completely non-toxic. While reference repellant, DEET also show non-toxicity. Azadirachtin (from Neem leaf) was highly immunotoxic (as shown in Table 8).

https://doi.org/10.55549/zbs.1417938

Table 8: Toxicity prediction/ probability of Terpinene, Azadirachtin and DEET (obtained using Protox II webserver).

Target	Toxicity prediction/ probability		
	Terpinene (Lemon peel)	**Azadirachtin (Neem leaf)**	**DEET (Reference repellant)**
Hepatotoxicity	Inactive (0.78)	Inactive (0.87)	Inactive (0.95)
Carcinogenicity	Inactive (0.75)	Inactive (0.50)	Inactive (0.86)
Immunotoxicity	Inactive (0.95)	**Active (0.99)**	Inactive (0.99)
Mutagenicity	Inactive (0.85)	Inactive (0.69)	Inactive (0.94)
Cytotoxicity	Inactive (0.85)	Inactive (0.52)	Inactive (0.75)

Drug-likeness of lead components and reference

The flexibility, insolubility, insaturation, lipophilicity, size and polarity of lemon peel (Terpinene), neem leaf (Azadirachtin) and the reference repellant (DEET) represented within the pink region of the physicochemical space hexagon (Figure 2) revealed that Terpinene and reference DEET fall entirely within the drug-likeness space while Azadirachtin failed due to its size, flexibility and polarity which extended beyond the region. This infers that lemon peel is comparable with DEET while performing better than Azadirachtin.

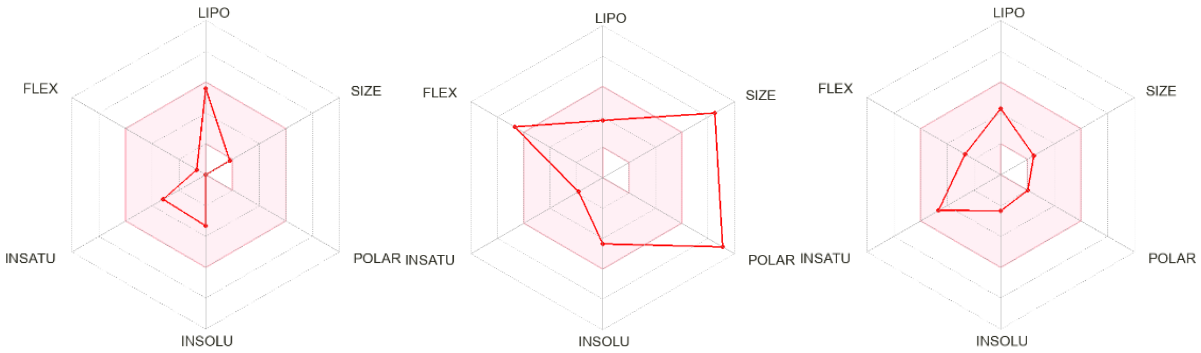

Figure 2: Hexagonal physicochemical space displaying drug-likeness of Terpinene, Azadirachtin and repellant reference DEET.

Lead components and reference' inhibition/ substrate capacity

The drug likeness/ bioactivity scores of Terpinene, Azadirachtin and repellant reference DEET is presented in Figure 3. For an average organic molecule, the bioactivity score when greater than 0.00 is defined as active, −0.50 to 0.0 is moderately active, and if less than −0.50 then inactive. From the diagram, it is clear that Terpene is moderately active as ion channel modulator (ICM), nuclear receptor ligand (NRL) and enzyme inhibitor (EI) with values ranging from -0.2 - -0.3. It is also evident that reference DEET are moderately bioactive as ion channel modulator (ICM) and enzyme inhibitor (EI) with values ranging from -0.4 - -0.5. Azadirachtin was found to be only moderately active as protease inhibitor (PI) with inhibitory values -0.35. The diagram also shows that Azadirachtin is highly inactive against ion channel modulator

https://doi.org/10.55549/zbs.1417938

(ICM) while Terpene also showed inactive tendency towards kinase inhibitor (KI) and protease inhibitor (PI) with values far less than -0.5.

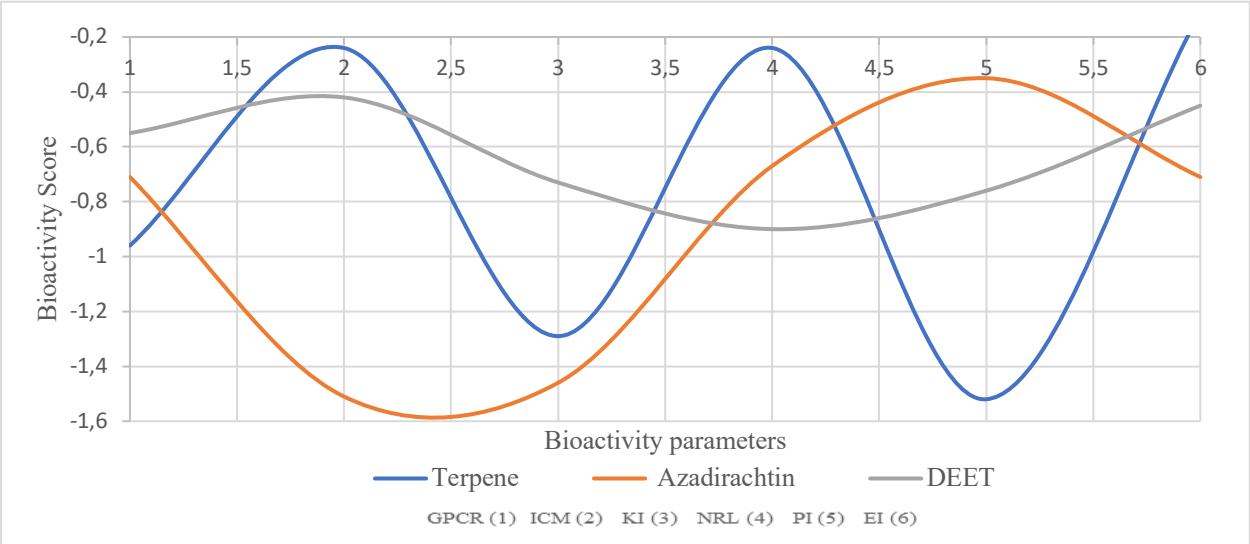

Figure 3: Molinspiration bioactivity score of Terpinene, Azadirachtin and repellant reference DEET revealing inhibition/substrate capabilities.

Binding interactions of lead components and reference repellant with *P. falciparum*

The lead compounds; Terpinene showed moderate π stacking, alkyl and π-alkyl binding interactions with Phe_{109}, Leu_{30}, Phe_{111}, Tyr_{112}, Ile_{107}, Ile_{80} and Met_{104}; while Azadirachtin, though fewer, showed hydrogen bond and π-sigma interactions with Leu_{8} and Ser_{75}, and Phe_{111} amino acid residues of histo-aspartic protease of *Plasmodium falciparum* respectively. 3-D structure of the reference repellant DEET revealed that Terpene from Lemon peel compares considerably as the reference showed moderate interactions with amino acid residues such as Ile_{107} (π-alkyl), Ile_{84} (π-sigma), Leu_{73} (π-sigma), Phe_{109} (π stacking), Tyr_{112} (π-sigma), Val_{120} (π-sigma), His_{32} (hydrogen bond) and Trp_{39} (π-sigma) (Figure 4A1, B1 and C1).

The bond length ranges from 2.5 to 5.2 Å, while the interacting residues showed moderate solvent accessibility in Terpene and DEET, Azadirachtin revealed better solvent accessibility surface (Figure 4A2, B2 and C2). Notably, the two lead components showed excellent hydrophobicity (entirely brown surface) while the reference DEET revealed some hydrophilic surface interactions alongside its comparable hydrophobicity (Figure 4A3, B3 and C3).

Table 9: Summary of docking studies/ binding interaction of Terpinene, Azadirachtin and DEET with histo-aspartic protease (HAP) zymogen residues from *Plasmodium falciparum* (using Pyrx 0.8 and Discovery studio 2020 softwares).

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
3qvc_Terpene_uff_E=101.04	-6.6	0	0
3qvc_ Azadirachtin_uff_E=3103.05	-7.3	0	0
3qvc_DEET_uff_E=273.66	-6.5	0	0

https://doi.org/10.55549/zbs.1417938

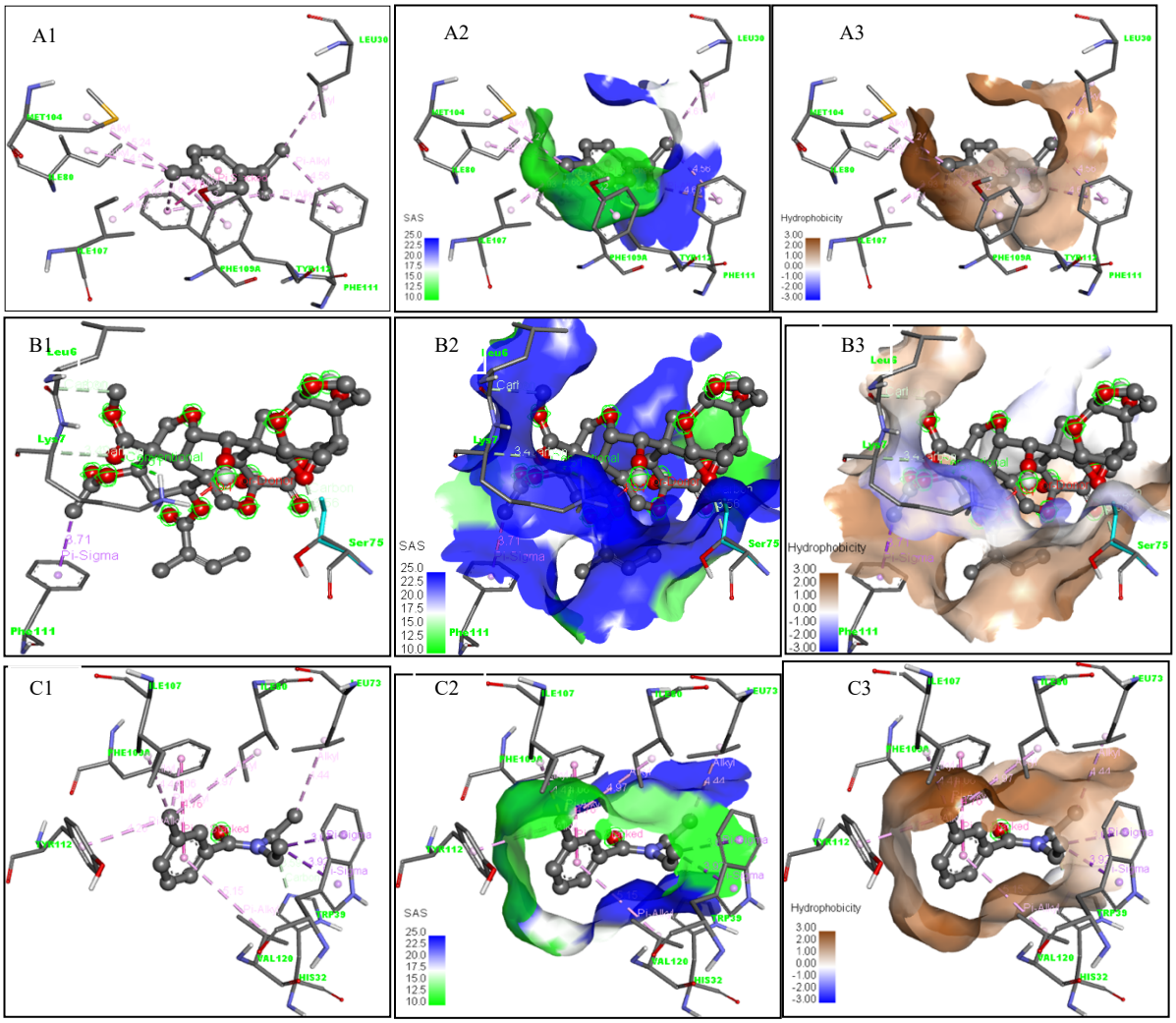

Figure 4: 3-D structures showing enzyme-ligand, solvent accessibility and hydrophobic interactions of Terpinene (A1-A3), Azadirachtin (B1-B3) and DEET (C1-C3) with histo-aspartic protease (HAP) zymogen residues from *Plasmodium falciparum*

Conclusion

In conclusion, all the extracts could be developed commercially as an effective personal protection measure against mosquito bites and to control diseases caused by mosquito-borne pathogen.

https://doi.org/10.55549/zbs.1417938

REFERENCES

Alison and James (2001). Toxic effects of Diethyltoluarnide and Dimethylsulphate creams as mosquito repellents on rabbi's skin. Journal of Anatomical Society of India.; 50 (2): 148 - 152)

Andrew, M.W. (1983). Agricultural innovation in the Early Islamic World: The Diffusion of Crops and Farming Techniques, Cambridge University Press 260pp.

Brown H.F. (2002). The Royal Horticultural Society New Encyclopedia of Herbs and their Uses Kindersley Publishers, United Kingdomn 350pp

Bruneton, J., (1999). Pharmacognosy, Phytochemistry and Medicinal Plants. Intercept Ltd., England, U.K. 250pp

Curtis CF, John E.F. and Anderson E.F (1989). Natural and Synthetic repellents. In Appropriate technology in vector control; pp: 75 - 92.

Egwaikhide, P.A. and C.E. Gimba, (2007). Analysis of the phytochemical content and anti-microbial activity of Moringa Journal. Sci. Res., 2(3-4): 135- 138

Harborne, J.B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis: 3rd (Ed.), Chapman and Hall, New York.250pp

Odetola, A.A. and O. Bassin, (1986). Evaluation of Antimalarial Properties of Some Nigerian Medicinal Plants, Research in Nigeria. University Press, Ibadan.350pp

Onaijobi, FD., (1986). Smooth muscle contracting lipid soluble principles in chromatographic fractions of Ocimum Gratissimum. Journals of. Ethnopharm., 18: 3- 11.

Oshaghi, MA., (2003). Repellent Effects of Extracts and Essential Oils of Citrus limon (Rutaceae) and (Labiatae) Against Malaria Vector, Anopheles, Iranian Journal of Public Health: 32 (4): 47-52.

John, B.C, Michael E.T (1998). Pharmacokinetics, formulation, and safety of insect repellent DEET: A review, Journals of malaria Control Assoc. 14:12-27

Ranson E.L. (200 1). Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector Anopheiles gambiae. Biochemical Journal. 359 :295 - 304.

Schreck CE, McGovern T.P. (1989) Repellents and other personal protection strategies against Aede albopictus. J. American Mosquito Control Association 247-252.

Seyoum, A. (2002). Repellency of five potted plants against Anopheles gambiae from human balts in semi-field host odours. Medical and Veterinary Entomology; 76: 239 - 45.

https://doi.org/10.55549/zbs.1417938

Sofowara, A., (1995). Medicinal Plants and Traditional Medicines in Africa. Chichester Sohn, Willey and Sons, New York, pp: 256.

WHO (1996). Informal Consultation: a rapid dipstick antigen capture assay for the diagnosis of faciparum malaria. Bulletin of the World Health Organization: 74 (1): 47- 54.

Jain, N., & Sharma, M. (2017). Evaluation of essential oil for its chemical and biological properties against fungi causing dermatophytic infection in human beings. *Analytical Chemistry Letters*, *7*(3), 402-409.

El-Sayed, A. S., George, N. M., Abou-Elnour, A., El-Mekkawy, R. M., & El-Demerdash, M. M. (2023). Production and bioprocessing of camptothecin from Aspergillus terreus, an endophyte of Cestrum parqui, restoring their biosynthetic potency by Citrus limonum peel extracts. *Microbial Cell Factories*, *22*(1), 4.

https://doi.org/10.55549/zbs.1417938