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Focus and Scope

The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

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Evaluation Of Computational And Insecticidal Activities Of Oils From Ocimum Gratissimum And Cymbopogon Citratus Against Anopheles Gambiae Mosquito

[1]Elelegu, EJ, [1]Dunkwu, CC, [1]Enyi, KC, [2]Onuelu, JE, [1]Onyesom, I.

ABSTRACT

Introduction: This study investigated the insecticidal and computational activities of essential oils from *Ocimum gratissimum* (*OgEO*), *Cymbopogon citratus* (*CcEO*), their combination (*OgEO+CcEO*), and permethrin against *Anopheles gambiae* mosquito stages.

Materials and Methods: Permethrin the standard chemical insecticide, demonstrated superior efficacy, with LC_{50} values of 0.1 ± 0.07 µg/mL (adulticidal), 0.001 ± 0.006 µg/mL (pupicidal), 0.005 ± 0.0003 µg/mL (larvicidal), and 0.01 ± 0.004 µg/mL (ovicidal), significantly lower than other treatments ($p < 0.05$). The *OgEO+CcEO* combination exhibited synergistic effects, with LC_{50} values of 4.5 ± 0.2 µg/mL (adulticidal), 5.0 ± 0.4 µg/mL (pupicidal), 1.6 ± 0.3 µg/mL (larvicidal), and 1.3 ± 0.2 µg/mL (ovicidal), outperforming individual oils ($p < 0.05$). *OgEO* was more effective than *CcEO*, with LC_{50} values of 5.7 ± 0.3 µg/mL (adulticidal) and 1.8 ± 0.5 µg/mL (ovicidal) compared to *CcEO*'s 6.3 ± 0.2 µg/mL and 5.1 ± 0.2 µg/mL, respectively. *In silico* study revealed strong binding of thymol (*OgEO*) to catalase (-5.14 docking score, -61.84 kCal/mol MMGBSA) and γ-muurolene (*CcEO*) to chorion peroxidase (-5.85 docking score, -74.65 kCal/mol MMGBSA), indicating disruption of key mosquito proteins involved in oxidative stress and eggshell formation.

Results: Pharmacokinetic analyses highlighted thymol's high gastrointestinal absorption and blood-brain barrier permeability, suggesting systemic toxicity, while γ-muurolene's high lipophilicity supports its suitability for topical or volatile applications. Both oils exhibited significant reduction in mosquito reproduction and enhancing their vector control potential.

Conclusion: Despite permethrin's unmatched efficacy, the *OgEO+CcEO* combination offers a promising eco-friendly alternative due to its synergy and lower environmental impact.

KEYWORDS: Mosquito-borne diseases, Essential oils, *Ocimum gratissimum, Cymbopogon citratus, Anopheles gambiae,* Insecticidal activity, Synergistic effects

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INTRODUCTION

Background to the Study

Mosquito-borne diseases remain a significant global public health challenge, causing substantial morbidity and mortality, particularly in tropical and subtropical regions. Among the most formidable culprits are malaria, dengue fever,

Zika virus, and chikungunya. According to the World Health Organization (WHO), malaria alone claimed about 597,000 lives in 2024, with the majority of deaths occurring in WHO Africa Region, particularly among pregnant women and children under the age of five. [1]. Malaria, caused by *Plasmodium* parasites and transmitted primarily by *Anopheles* mosquitoes, is one of the oldest and deadliest mosquito-borne diseases. The disease causes low birth weight, miscarriages or stillbirths, and infant death in sub-Saharan Africa. [2] There are around 500 species of *Anopheles* mosquitoes that serve as *Plasmodium* parasite vectors worldwide.[3] Mosquitoes are efficient vectors of various pathogens, making them responsible for the transmission of deadly diseases affecting millions of people worldwide. Despite extensive efforts to control and eradicate malaria, the disease continues to impose a heavy toll on human health and well-being, particularly in sub-Saharan Africa. [4]

Protection against mosquito bites is one way of being used to reduce illness prevalence. [5] [6] Treatment with antimalaria drugs, use of prophylactic drug, insecticidal treated bed-nets, removal of stagnant waters, among others have been employed in the treatment and control of mosquito-borne diseases. [7] Some of these strategies are expensive, not easily accessible to rural dwellers, produces discomfort and faced with the issue of resistance. [8] The extensive use of synthetic insecticides in recent times for mosquito control has led to the development of resistance in mosquito populations. [8] Mosquitoes can rapidly evolve resistance mechanisms, rendering widely-used insecticides ineffective and limiting the options available for disease control.[9] The WHO has identified insecticide resistance as a major obstacle to malaria control and eradication efforts. [10] Chemical insecticides are not only hazardioushazardous to human and animal health but they are also toxic to the environment and their bio-accumulation in the environment results in ecosystem imbalance.[7]. In the face of these challenges, there has been a growing interest in exploring natural alternatives for mosquito control. Essential oils derived from various plant species have shown promise as effective and environmentally friendly mosquito control agents.[11] Presently, insecticidal properties of different plants including scent leaf and lemon grass have been the subject of intense research. [6][12]

Scent leaf (*Ocimum gratissimum*) is a tropical plant with a long history of use in traditional medicine and culinary practices. Studies have indicated the potential larvicidal and adulticidal activities of essential oil extracted from scent leaf against mosquito vectors. [13] Also, lemon grass (*Cymbopogon citratus*), another plant with diverse applications, has also exhibited mosquitocidal properties. Nguyen *et al.* [14] conducted a study highlighting the larvicidal activity of *Cymbopogon citratus* essential oil against *Anopheles gambiae*, a major vector of malaria. Hence, this research aims to assess the synergistic mosquitocidal activity of scent leaf and lemon grass oil across the life cycle stages of *Anopheles gambiae* mosquito.

MATERIALS AND METHODS

Materials

The materials used in this study included; fresh leaves of *Ocimum gratissimum* and *Cymbopogon citratus*, Clevenger-type essential oil distillation unit (Borosil Glass Works Ltd., India), heating mantle (MF500), distilled water, white enamel rearing bowls, glass jars with conical gauze nets, mosquito netting cages, pipettes, plastic containers, yeast, Biological Oxygen Demand (BOD) incubator (Heracell 150i MD), 10 % glucose solution, 20 % glucose solution,

compound microscope (Swift SW380T), universal containers, Permethrin among others.

Collection and Authentication of Plants

Ocimum gratissimum (Scent leaf) and *Cymbopogon citratus* (Lemon grass) plants were collected in Abraka, in Ethiope East L.G.A. of Delta State, Nigeria. Abraka (Longitude: 6° 04'E, Latitude**:** 5° 54'N) has a tropical wet and dry climate, with a lengthy wet season and relatively constant temperatures throughout the year. The plants were identified by Leaf Snap App at the point of collection and authenticated in the Herbarium Unit, Department of Botany, Delta State University, Abraka. Fresh leaves of *Ocimum gratissimum* with Voucher number: DELSUH-196 and *Cymbopogon citratus* with Voucher number: DELSUH -244 were then taken to Department of Pharmacology and Toxicology, Faculty of Pharmacy, Delta State University, Abraka where the study was carried out. The leaves were washed with running tap water to remove dust particles and debris from their surfaces, and thereafter, allowed to air dry for 21 days at room temperature (23-31 ° C) and pulverized for further analysis [12]

Oil Extraction by Hydro distillation

Hydro distillation was carried out in a Clevenger apparatus (Clevenger-Type Essential oil Distillation Unit, Borosil Glass Works Ltd., India) according to the methods of Sadgrove and Jones [15]. About 1kg each of *gratissimum* and *citratus* sample was directly immersed in 10 L of distilled water. The solid-liquid mixture was heated until boiling under atmospheric pressure using heating mantle (MF500). The volatile substance present in the plant sample evaporated along with the steam generating column by the water. This azeotropic mixture was then condensed in the condensing column of the Clevenger apparatus (Clevenger-Type Essential oil Distillation Unit, Borosil Glass Works Ltd., India) and separated by its density difference and immiscibility. The essential oil collected was stored in universal container at room temperature for use.

Mosquitocidal Test

Mosquito rearing, maintenance and identification

Rearing of mosquito in the laboratory is of primary importance in the maintenance of a mosquito colony. These laboratory maintained colonies are by themselves of great relevance as they readily provide sufficient number of mosquitoes, both aquatic and adult stages, for insecticide bioassays [14].

The materials and methods for rearing of mosquitoes as described by Onyido *et al.* [16] were adopted for this study. This involved the use of yeast for *Anopheles* larvae which are surface feeders. A rearing bowl (white enamel pan 30 cm x 12.5 cm) was placed outside for about 24-48 hours to obtain about 500 1st instar larvae of the *Anopheles*, a measured quantity of 0.5g of yeast had been found to be suitable. This technique was developed out of necessity for rearing higher numbers of adult mosquitoes. Distilled water was used as it does not require aeration at any stage of the larval growth.

For the adult maintenance, as the pupae emerged from the larvae, they were hand-picked with the aid of pipette into a glass jar covered with a conical gauze net about 16 cm in diameter and about 19 cm high, with an opening at the apex about 2.5 cm in diameter. This cone prevent the adult from drowning in the water of the pupal bowl and the females from laying the eggs into the pupal bowl, but rather in the special egg bowl prepared and provided for them. The cage also was provided with 10 % solution of glucose on which males and females that had not taken blood meals could feed. This container was in the form of a 7.5 cm x 2.5 cm tube inverted in another tube

slightly larger in diameter and 5 cm longer. The smaller one was covered with a piece of lint and the large diameter tube filled with 20 % glucose solution. The lint acted like a wick and remained damp with glucose solution. The wick was washed twice daily, and the glucose solution also renewed twice a week. Like humans, mosquitoes have need for space. As a result some mosquitoes could mate in the laboratory cage while others cannot. One 30 cm^3 cage of mosquito nettings or gauze on a wooden frame was well fitted for the maintenance of those species of mosquito which would mate in captivity. For ordinary day-to-day colony maintenance, the ideal number of adult mosquitoes per cage of this size were 500 mosquitoes (approximately 250 males and 250 females). This ensured adequate mating and egg production for perpetuation of the colony. Larger numbers lead to high mortalities and smaller number to inadequate fertilization of the females. Also, the insects were maintained in a biological oxygen demand (BOD) incubator (Heracell 150i MD), under controlled conditions of temperature 27 ± 2 °C, relative air humidity 75 ± 5, and a 12-hour light and dark photoperiod.

These reared mosquitoes were then identified to species level using taxonomic keys and morphological characteristics. Experienced entomologists from the department of Animal and Environmental Biology, Delta State University, Abraka, employed microscopes to examine key morphological features of the mosquitoes for accurate identification.

Ovicidal activity

Ovicidal efficacy bioassays were carried according to the methods of Obiang *et al.* [17]. One mL of the essential oils of *Ocimum gratissimum, Cymbopogon citratus* and combine form of both (*Ocimum gratissimum* and *Cymbopogon citratus*) at concentration of 1, 2, 3, 4, 5 and 10 % prepared with the appropriate volume of distilled water in six plastic cups (115 mm diameter and 80 mm depth). Then, 20 recently-laid eggs were held in the insectary, and the egg mortality was recorded at 0, 60, 120 and 300 sec post-treatment. The experiment was conducted in duplicates.

Larvicidal assay

A larvicidal assay was conducted to evaluate the efficacy of the oils against *An. gambiae* larvae according to the method outlined by Antonio-Nkondjio *et al.* [18]. This assay aimed to assess the ability of the oils to kill or inhibit the development of *An. gambiae* larvae, thereby disrupting their life cycle and reducing mosquito populations. Experimental studies were carried out using standardized protocols to determine the larvicidal activity of the oils. Various concentrations (1, 2, 3, 4, 5 and 10 %) of the oil were applied to the larvae, and mortality rates were monitored at 0sec, 60 sec, 120 sec and 24 hrs. Permethrin was used as control to compare the efficacy of the oils against untreated larvae. The experiment was conducted in duplicates

Pupicidal activity

Pupicidal activity was assessed according to the protocol provided by Fernandes *et al.* [19]. Twenty pupae of *Anopheles gambiae*, with a maximum of 24 of life, were placed in a plastic container with 10 mL of the oily solution at different concentrations (1, 2, 3, 4, 5 and 10 %). Pupae mortality/ mosquito emergence were verified after 0sec to 5min, 60 mins, 24 hrs, 48 hrs and 72hrs of exposure. The experiments was performed in a BOD incubator (Heracell 150i MD) as described above. The negative and positive controls were maintained, and the duplicates were assessed, as described previously for the ovicidal test.

Adulticidal activity

The protocols which was used to evaluate the effect of the oils on adult mosquitoes, were adopted from Nunes *et al.* [20]. The walls of the plastic containers was moistened with the oily solutions at various concentrations (1, 2, 3, 4, 5 and 10 %) and allowed to dry. Twenty *An. gambiae* mosquitoes (5–6 days of emergence) were placed in these containers, and the mortality was checked at 0sec to 5min, 60 mins, 24 hrs, 48 hrs and 72hrs. The experiment was conducted in duplicates. This method replicate the method of indirect application of insecticides on surfaces and allow the evaluation of their residual effect.

In silico study

In the *In silico* study, target proteins crucial for insecticidal activities in mosquitoes were docked with the retrieved compounds of the plants' essential oils. The focus was, therefore, on insecticidal proteins (Aquaporins-3, Catalase, 3-Hydroxykynurenine transaminase, carbonic anhydrase, Arylalkylamine, N-Acetyl-transferase, Chorion peroxidase, V-ATPase, and Phosphofructokinase). As well as insect resistance reversal proteins (Cytochron p450 monoxygenases, Glutathione S-transferases). [21] *In silico* study employed to determine the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the essential oils include OSIRIS tools.

Retrieval and Preparation of Sterilants, insecticide resistance, and Insecticidal activity protein Targets

The three dimensional structures of flight inhibition targets; Ornithine decarboxylase [UniProt ID: A0A1S4H8Q3], Catalase [UniProt ID: Q6RBZ5], Heme oxygenase [UniProt ID: Q7Q288], Vitellogenin 2 [UniProt ID: Q9NAW8], ATP-dependent 6-phosphofructokinase [UniProt ID: A0A1S4GVQ5], V-type proton ATPase catalytic subunit A [UniProt ID: Q5TTG1], Catalase [UniProt ID: Q6RBZ5], Carbonic anhydrase [UniProt ID: Q6WLH6], 3-hydroxykynurenine transaminase [UniProt ID: Q7PRG3], Aquaporin [UniProt ID: Q7PWV1], Chorion peroxidase [UniProt ID: Q7QH73], and Glutathione S-transferase 1 [UniProt ID: Q93113] were downloaded from Alphafold and UniProt database and further prepared using Glide's protein preparation wizard. Missing protein residues (atoms and loops) which are essential to protein structures were fixed using loop refinement methods. Using energy minimization process, the protein structures were optimized to exclude steric clashes, refine conformation and improve geometry.

Ligand Retrieval and Preparation

Bioactive compounds of *Ocimum gratissimum* (commonly known as African basil) and *Cymbopogon citratus* (lemongrass) were obtained from PubChem Database and prepared using the LigPrep 2.4 software, which can generate a number of structures from each input structure with different ionization states, tautomers, stereochemistries, and ring conformations to eliminate molecules based on various criteria such as molecular weight or the number and type of functional groups present with correct chiralities for each successfully processed input structure. [22] The OPLS-2005 force field was employed for optimization, which resulted in the ligand's low-energy conformer.[23]

Binding Site Prediction and Receptor Grid Generation

SiteMap was used to generate binding site characteristics, enabling visualization in Maestro. It initiated with a search phase identifying potential binding regions on or near the protein surface, termed sites, using a grid of site points. Subsequently, contour maps are produced,

delineating hydrophobic and hydrophilic features, further categorized into donor, acceptor, and metal-binding regions. The evaluation phase assesses each site by computing various properties, integrated into the Maestro project upon completion. Using the best ranked sitemap, receptor grids were generated for each of the proteins using Receptor Grid Generation module embedded in maestro software suite. [24]

Receptor Based Virtual Screening

To evaluate the docking parameters, all potential compounds were docked into the protein targets using Schrodinger's Grid-Based Ligand Docking (Glide) software. [25] [26] Glide 5.6's Receptor Grid Generation module was used to define the active site for docking ligands. To investigate the binding modes of the compounds against individual targets, two distinct docking techniques were used, standard precision (SP) and extra precision (XP) docking, were carried out.

Prime MM/GBSA Calculations

The Prime/MM-GB/SA technique is used to compute the free energy of binding. This method is used to calculate the free energy of binding for a given collection of ligands to a receptor. The docked postures were reduced using Prime's local optimization function, and the complex energies were estimated using the Optimized Potentials for Liquid Simulations-All Atom (OPLS-AA) (2005) force field and the generalized-Born/surface area (GB/SA) continuum solvent model. The binding free energy, G bind, is computed as follows[26]:

$\Delta Gbind = \Delta E + \Delta Gsolv + \Delta GSA$ (1)

$\Delta E = Ecomplex - Eprotein - Eligand$ (2)

Where Ecomplex, Eprotein, and Eligand are the minimized energies of the protein–inhibitor complex, protein, and inhibitor, respectively

$\Delta Gsolv$ = Gsolv(complex)–Gsolv(protein)–Gsolv(ligand)

Where Gsolv(complex), Gsolv (protein), and Gsolv(ligand) are the solvation free energies of the complex, protein, and inhibitor, respectively:

ΔGSA = GSA (complex)–G SA(protein) – G SA(ligand)

Where GSA (complex), GSA (protein), and GSA (ligand) are the complex, protein, and inhibitor surface area energies, respectively.

Assessment of Pharmacokinetic (drug-likeness and ADM ET) and physicochemical properties

Promising hit compounds having good docking score, XP GScore, and MMGBSA dG Bind were selected for ADMET studies in addition to other physiochemical analysis using ADEMET lab 3.0 for further analysis. ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties are crucial parameters in drug discovery and development. Predicting the ADMET properties of small molecules is essential for optimizing drug design and development strategies, as well as for assessing the safety and efficacy of drug candidates.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.0 for Windows (GraphPad Software, San Diego, CA, USA). For the ovicidal, larvicidal, pupicidal and adulticidal assays, the significant differences between the groups were analyzed using one-way ANOVA and Tukey post-hoc test ($p < 0.05$). The LC_{50} werewas calculated using non-linear regression, considering a 95 % significance level.

RESULTS

Mosquitocidal Studies

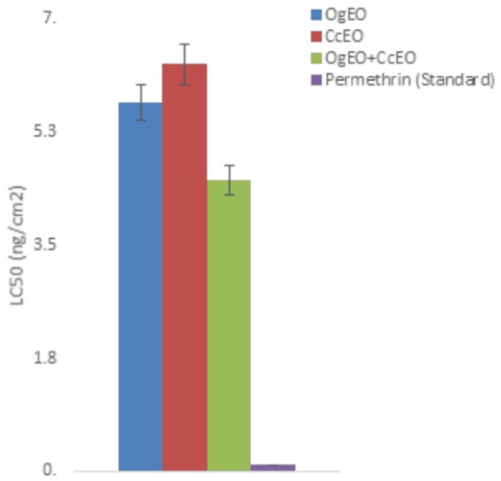
The Figures (4.1 a-d) present the lethal concentration (LC_{50}) values required to achieve 50 % mortality in *Anopheles gambiae* mosquitoes at

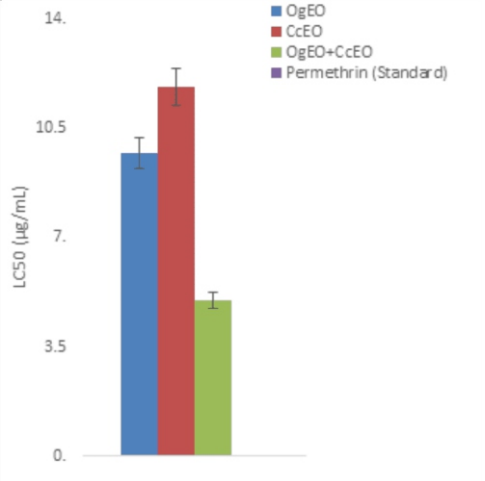
different life stages (ovicidal, larvicidal, pupicidal, and adulticidal) for four treatments: *Ocimum gratissimum* essential oil (*OgEO*), *Cymbopogon citratus* essential oil (*CcEO*), their combination (*OgEO+CcEO*), and permethrin (the standard insecticide). The LC_{50} values were reported as mean ± standard deviation.

OgEO alone is more effective than *CcEO*, with LC_{50} values of 5.7 ± 0.3 for adulticidal, 9.7 ± 4.0 for pupicidal, 4.1 ± 0.2 for larvicidal, and 1.8 ± 0.5 for ovicidal activities, compared with *CcEO*'s 6.3 ± 0.2, 11.8 ± 0.7, 5.3 ± 0.6, and 5.1 ± 0.2, respectively. *OgEO*'s lower LC_{50} values were statistically significant ($p < 0.05$) compared with *CcEO*, indicating greater potency. The ovicidal activity of both *OgEO* and the combination suggests some statistical overlap, but *OgEO*'s LC_{50} (1.8 ± 0.5) is still significantly lower than *CcEO*'s (5.1 ± 0.2), indicating better ovicidal activity.

CcEO is the least effective among the essential oils, with the highest LC_{50} values in most stages, and its ovicidal LC_{50} (5.1 ± 0.2) activity is significantly higher than that of permethrin, *OgEO*, and the combination ($p < 0.05$). The pupicidal activities of *OgEO* and *CcEO* suggest that their LC_{50} values (9.7 ± 4.0 and 11.8 ± 0.7, respectively) may not differ significantly from each other in this stage, although, both were significantly less effective than the combination and permethrin.

Statistically, data showed that the LC_{50} values for each treatment were significantly different from one another within each life stage ($p < 0.05$). The low standard deviations for most LC_{50} values (i.e., 0.07 for permethrin in adulticidal, 0.2 for the combination in larvicidal) suggest high precision in the measurements, reinforcing the reliability of the observed differences. However, the higher standard deviation for *OgEO* in pupicidal activity (9.7 ± 4.0) indicates greater variability, which may warrant further investigation into its consistency in this stage.





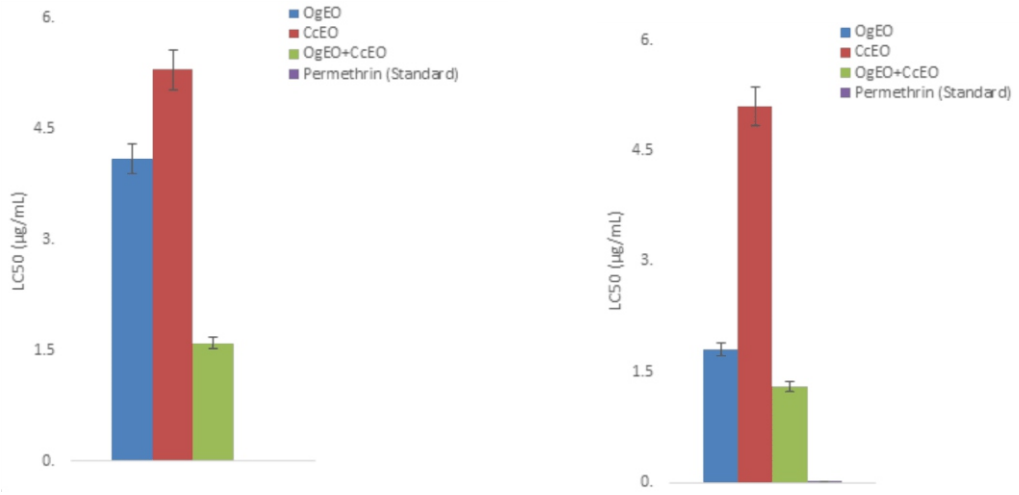


Figure 4.1: Adulticidal (a) and Pupicidal (b) efficacy (LC_{50}) of *Ocimum gratissimum* essential oil (*OgEO*), *Cymbopogon citratus* essential oil (*CcEO*), their combination (*OgEO+CcEO*), and permethrin. Lavicidal (c) and Ovicidal (d) efficacy (LC_{50}) of *Ocimum gratissimum* essential oil (*OgEO*), *Cymbopogon citratus* essential oil (*CcEO*), their combination (*OgEO+CcEO*

In silico study for target proteins crucial for insecticidal activities

The 17 (camphene, β-caryophyllene, α- and β-pinene, α-humulene, sabinene, β-myrcene, limonene, 1,8-cineole, trans-β-ocimene, linalool, α- and -terpineol, eugenol, α-copaene, β-elemene, p-cymene, thymol, carvacrol) and 12 (6-methylhept-5-en-2-one, camphene, limonene, nonan-4-ol, citronellal, neral, geranial, citral, geranylacetate, β-caryophyllene, γ-muurolene, caryophyllene oxide) compounds, respectively obtained from the essential oils of Ocimum gratissimum and cympopogon citratus and retrieved from pub database were docked against 9 proteins (aquaporin 3, catalase, 3-hydroxykynurenine transaminase, carbonic anhydrase, arlalkylamine, N-acetyltransferase, chorion peroxidase, V-ATPase, phosphofructokinase) essential for Anopheles gambiae mosquito survival, and so served as insecticidal targets. The results obtained are displayed in Figures 4.2 – 4.4.

Figure 4.2 showed the binding data of thymol (PubChem ID: 6989) from O. gratissimum with catalase and γ-muurolene (PubChem ID: 12313020) from C. citratus with chorion peroxidase (UniProt ID: Q7QH73). These compounds produced the strongest binding among the compounds in O. gratissimum and C. citratus against the docked targets.

The visual data on two key aspects of binding interactions interactions (bond type and amino acid residue interactions) between thymol and catalase, and then, γ-muurolene and chorion peroxidase are shown in Figure 4.3 a-d.

The 2D interaction complexes between thymol and catalase, and then, γ-muurolene and chorion peroxidase are shown in Figures 4.4.

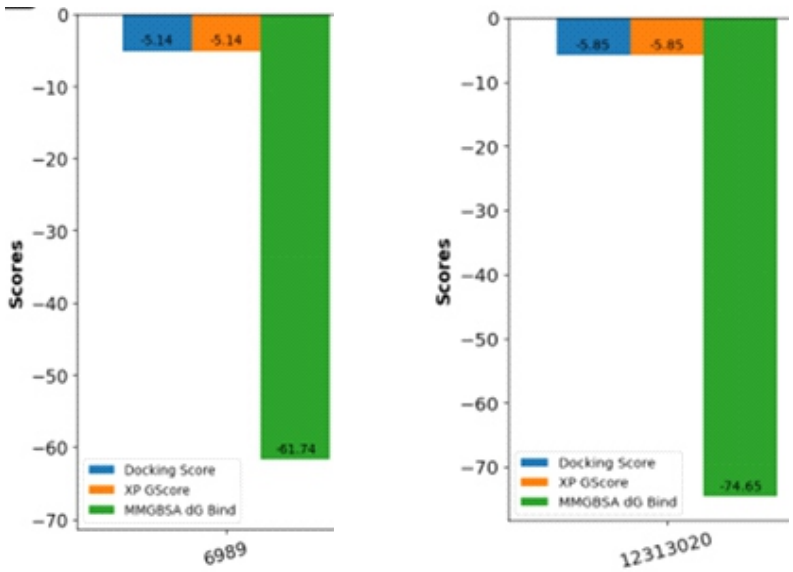


Figure 4.2: Docking scores of thymol (PubChem ID: 6989) against catalase (UniProt ID: Q6RBZ5), (A), and γ-muurolene (PubChemID: 12313020) with chorion peroxidase (UniProt ID: Q7QH73), (B).

The docking scores of the best two compounds from Ocimum gratissimum (thymol) and Cymbopogon citratus (γ-muurolene) against two different proteins are presented in Figure 4.5 In A, catalase (UniProt ID: Q6RBZ5), binding with thymol, showed a docking score of -5.14, XP GScore of -5.14 also, with an MMGBSA dG Bind of -61.84 kCal/mol, and an aggregate score of -1.79. However in B, the stronger interaction was with chorion peroxidase (Q7QH73), where γ-muurolene achieved a docking score of -5.85, an XP GScore of -5.85, and an MMGBSA binding energy of -74.65 kCal/mol with a normalized aggregate score of -2.08.

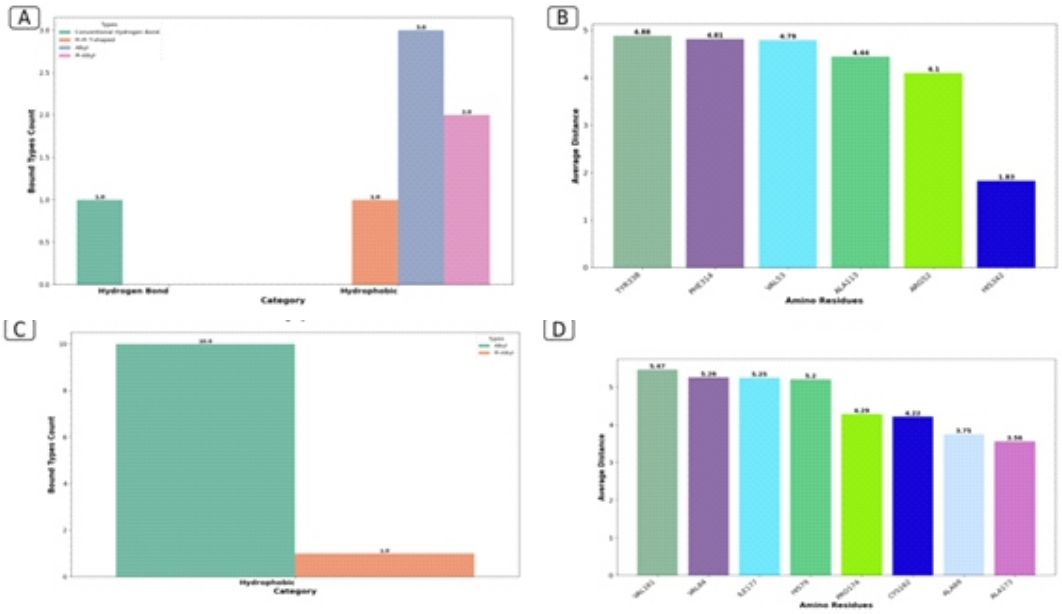


Figure 4.3: The binding interactions between Thymol and Catalase (A and B) and γ - Muurolene and Chorion peroxidase (C and D). On [A], the bond type distribution shows one hydrogen bond and six hydrophobic interactions, specifically consisting of three Pi-Alkyl, two Alkyl, and one Pi-T-shaped bond. On [B], the average distances of these bonds with various amino acid residues are displayed. Tyr 338 and Phe 314 form the longest bonds (~4.88 Å and 4.81 Å), while His 342 forms the shortest bond (1.83 Å), indicating a strong interaction. This suggests that hydrophobic interactions dominate the binding of thymol, with hydrogen bonding playing a minor but important role in stabilizing the complex, especially through the interaction with His 342.

On C, the binding interactions of γ-Muurolene from *Cymbopogon citratus* with Chorion peroxidase (UniProt ID: Q7QH73) ar\e dominated by hydrophobic interactions, with 10 alkyl bonds and 4 pi-alkyl. On D, regarding the amino acid residues involved, Leu 589 had the longest average bond distance at 5.49 Å, followed by Phe 135 (5.08 Å), Phe 534 (5.01 Å), and Ile 629 (4.89 Å). The shortest bond distances are observed with Val 532 (4.14 Å), His 529 (4.33 Å), and Phe 594 (4.35 Å). These distances indicate that Leu 589 plays a key role in binding, with strong hydrophobic interactions throughout the site.

Figure 4.4: 2D Binding interaction complexes between thymol and catalase (A), and then, γ-Muurolene and chorion peroxidase (B).

4.3 Pharmacokinetic and Physicochemical Studies

As shown in Table 4.1, Thymol: exhibits high gastrointestinal absorption and blood-brain barrier permeation. It is moderately lipophilic, does not interact with P-glycoprotein, but inhibits CYP1A2. It shows good drug-likeness with minimal rule violations and easy synthetic accessibility. Gamma-Muurolene: Highly hydrophobic with low gastrointestinal absorption and no blood-brain barrier permeation. It inhibits CYP2C19 and CYP2C9, which suggests possible metabolic interactions. It has moderate drug-likeness but higher synthetic complexity. Ursolic Acid: A large, highly lipophilic molecule with significant polar interaction potential. It shows low gastrointestinal absorption and is not BBB permeant. Despite higher bioavailability score (0.85), it has multiple rule violations and high synthetic difficulty.

Thymol, with one hydrogen bond acceptor and donor each, has a high MR (48.01) and TPSA (20.23), indicating more potential for polarity. Its iLOGP (2.32) and XLOGP3 (3.3) suggest moderate lipophilicity. Thymol exhibits high GI absorption and is also BBB permeant. It does not act as a Pgp substrate, but inhibits CYP1A2, indicating potential metabolic interactions. Thymol does not inhibit other key CYP enzymes. Its log Kp (-4.87 cm/s) points to low skin permeability. Thymol has no violations of Lipinski's rules, but one violation of Ghose, with a bioavailability score of 0.55 and one lead-likeness violation. The synthetic accessibility score is relatively low at 1.

Gamma-muurolene, with no hydrogen bond acceptors or donors, has a relatively high MR (69.04) and no TPSA, indicating strong hydrophobicity. It is characterized by higher lipophilicity, with iLOGP of 3.31 and XLOGP3 of 4.31. Gamma-muurolene has low GI absorption and is not BBB permeant. It is neither a Pgp substrate nor an inhibitor of most CYP enzymes, but does inhibit both CYP2C19 and CYP2C9. Its log Kp (-4.49 cm/s) suggests moderate skin permeability. It has one violation of Lipinski's rule and no violations of Ghose or other rules. With a bioavailability score of 0.55. Gamma-muurolene also has two lead-likeness violations and a synthetic accessibility score of 4.35.

Table 4.1: Pharmacokinetic and Physicochemical Studies

Parameter	Thymol	Gamma-Muurolene
HBA / HBD	1 / 1	0 / 0
Molecular Refractivity (MR)	48.01	69.04
TPSA	20.23	0
iLOGP / XLOGP3	2.32 / 3.3	3.31 / 4.31
GI Absorption	High	Low
BBB Permeant	Yes	No
Pgp Substrate	No	No
CYP Inhibition	CYP1A2	CYP2C19, CYP2C9
log Kp (cm/s)	-4.87	-4.49
Lipinski Violations	0	1
Ghose Violations	1	0
Other Rule Violations	None	None
Bioavailability Score	0.55	0.55
Lead-likeness Violations	1	2
Synthetic Accessibility	1.00	4.35

Discussion

The results of this study provide significant insights into the insecticidal and computational activities of essential oils derived from *Ocimum gratissimum* (*OgEO*) and *Cymbopogon citratus* (*CcEO*), their combination (*OgEO+CcEO*), and permethrin (control) against *Anopheles gambiae* mosquitoes across various life stages (ovicidal, larvicidal, pupicidal, and adulticidal). Additionally, *In silico* studies elucidate the molecular interactions of key phytoconstituents, thymol (from *O. gratissimum*) and γ-muurolene (from *C. citratus*), with critical mosquito proteins, offering a mechanistic understanding of their insecticidal potential.

The results indicate that permethrin (the control), a synthetic pyrethroid, is the most effective treatment across all life stages of *Anopheles gambiae*, with significantly lower LC50 values (e.g.,

0.1 ± 0.07 µg/mL for adulticidal activity) compared to the essential oils and their combination ($p < 0.05$). This aligns with its well-documented neurotoxic mode of action, which disrupts sodium channels in insect neurons, leading to rapid paralysis and death. [27] The low standard deviations in permethrin's LC_{50} values (e.g., 0.07 for adulticidal, 0.0003 for larvicidal) suggest high precision and consistency, reinforcing its reliability as a standard insecticide. However, the environmental persistence and non-target toxicity of pyrethroids, as noted by Schleier and Peterson [28], highlight the need for eco-friendly alternatives, such as essential oils.

The combination of *Ocimum gratissimum* (*OgEO*) and *Cymbopogon citratus* (*CcEO*), demonstrated significantly greater efficacy than either oil alone ($p < 0.05$), particularly in larvicidal (LC_{50} = 1.6 ± 0.3 µg/mL) and ovicidal (LC_{50} = 1.3 ± 0.2 µg/mL) activities. This suggests a synergistic interaction between the phytoconstituents of the two oils, likely due to complementary modes of action. For instance, the combination's lower LC_{50} values compared with individual oils indicate enhanced bioactivity, possibly through multi-target effects on mosquito physiology. This finding corroborates studies by Pavela *et al.* [29], who reported synergistic effects in essential oil blends, particularly those containing monoterpenes and sesquiterpenes, against *Aedes aegypti* larvae. The synergy observed here could be attributed to the diverse chemical profiles of *Ocimum gratissimum* essential oil (rich in thymol and eugenol) and *Cymbopogon citratus* essential oil (rich in citral and γ-muurolene), which may disrupt multiple biochemical pathways in mosquitoes, such as respiratory, nervous, or enzymatic systems.

Individually, *OgEO* outperformed *CcEO* across most life stages, with significantly lower LC_{50} values (e.g., 1.8 ± 0.5 µg/mL for ovicidal activity vs. 5.1 ± 0.2 µg/mL for *CcEO*, $p < 0.05$). The higher potency of *OgEO* may be linked to its thymol content, a phenolic monoterpene known for its strong insect-repellent and toxic properties as reported by Tabari *et al.* [30]. *CcEO*'s relatively higher LC50 values, particularly in ovicidal activity, suggest lower efficacy, possibly due to its primary constituents (e.g., citral, γ-muurolene) having less potent interactions with mosquito targets. However, the pupicidal activity of both oils showed higher variability (e.g., *OgEO*: 9.7 ± 4.0 µg/mL), indicating potential inconsistencies in their efficacy at this stage, which warrants further investigation.

The *In silico* docking studies provided a mechanistic basis for the observed insecticidal activities. Thymol (from *OgEO*) and γ-muurolene (from *CcEO*) exhibited strong binding affinities to catalase (UniProt ID: Q6RBZ5) and chorion peroxidase (UniProt ID: Q7QH73), respectively, with docking scores of -5.14 and -5.85, and MMGBSA binding energies of -61.84 and -74.65 *kCal/mol*. These enzymes are critical for mosquito survival, with catalase detoxifying reactive oxygen species and chorion peroxidase contributing to eggshell formation. The strong binding of thymol to catalase, driven by one hydrogen bond (His 342, 1.83 Å) and six hydrophobic interactions (e.g., Tyr 338, Phe 314), suggests inhibition of oxidative stress management, potentially leading to cellular damage in mosquitoes. Similarly, γ-muurolene's interaction with chorion peroxidase, dominated by 14 hydrophobic interactions (e.g., Leu 589, 5.49 Å), may disrupt eggshell synthesis, explaining the oils' ovicidal efficacy.

These findings are consistent with Ugbogu *et al.* [31], who reported that monoterpenes like thymol target multiple insect proteins, including acetylcholinesterase and cytochrome P_{450} enzymes, disrupting neural and metabolic functions. The dominance of hydrophobic interactions in both compounds' binding profiles aligns with studies by Regnault-Roger *et al.* [32], who noted that lipophilic terpenoids penetrate insect cuticles more effectively, enhancing toxicity. The

stronger binding of γ-muurolene to chorion peroxidase compared with thymol's interaction with catalase suggests that *CcEO*'s ovicidal activity, although, weaker overall, may involve specific disruption of eggshell formation, complementing *OgEO*'s broader toxicity.

The PK/PD profiles of thymol and γ-muurolene provide insights into their potential as insecticidal agents. Thymol's moderate lipophilicity (iLOGP = 2.32, XLOGP3 = 3.3), high gastrointestinal (GI) absorption, and blood-brain barrier (BBB) permeability suggest good bioavailability and potential systemic effects in insects. Its inhibition of CYP1A2 indicates possible metabolic interactions, which could enhance its persistence in mosquito tissues. In contrast, γ-muurolene's high lipophilicity (iLOGP = 3.31, XLOGP3 = 4.31) and low GI absorption suggest it is better suited for topical or volatile applications, such as repellents. Its inhibition of CYP2C19 and CYP2C9 further supports its metabolic interference potential, which may contribute to toxicity.

Thymol's compliance with Lipinski's rules and low synthetic accessibility score (1) indicate its feasibility for large-scale production, whereas γ-muurolene's higher synthetic accessibility score (4.35) and two lead-likeness violations suggest challenges in formulation. These properties align with findings by Isman [33], who noted that monoterpenes like thymol are more readily absorbed and metabolized in insects compared with sesquiterpenes like γ-muurolene, which may accumulate in cuticle layers, enhancing contact toxicity.

The superior efficacy of permethrin in this study is consistent with findings by Norris *et al.* [34], who reported LC_{50} values of 0.08–0.12 μg/mL for permethrin against *Anopheles gambiae* adults, closely matching our results (0.1 ± 0.07 μg/mL). However, the environmental and health concerns associated with pyrethroids, as discussed by Tang *et al.* [35], underscore the value of essential oils as safer alternatives. The synergistic effect of *OgEO+CcEO* aligns with Benelli *et al.* [36], who found that blending *Ocimum* and *Cymbopogon* oils reduced LC_{50} values by 30–50 % against *Aedes* larvae compared with individual oils, supporting the hypothesis of multi-target synergy.

The ovicidal potency of *OgEO* (LC_{50} = 1.8 ± 0.5 μg/mL) is comparable with results by Govindarajan *et al.* [37], who reported an LC_{50} of 1.5 μg/mL for *O. basilicum* oil against *Anopheles stephensi* eggs, suggesting that *Ocimum* species share potent ovicidal compounds. The higher LC_{50} values for *CcEO* (e.g., 5.1 ± 0.2 μg/mL for ovicidal activity) are consistent with Mdoe *et al.* [38], who noted weaker larvicidal and ovicidal activities for *C. citratus* compared to other essential oils, likely due to lower concentrations of bioactive monoterpenes.

In silico studies by Kamaraj *et al.* [39] also support our findings, reporting strong binding affinities of thymol (-5.2 kCal/mol) to mosquito detoxifying enzymes, similar to our docking score of -5.14 for catalase. The hydrophobic interaction dominance in γ-muurolene's binding aligns with computational studies by da Silva *et al.* [40], who noted sesquiterpenes' efficacy against chorion-related proteins in *Culex* mosquitoes.

The significant efficacy of the *OgEO+CcEO* combination, particularly in larvicidal and ovicidal stages, suggests its potential as a natural insecticide for integrated vector management. However, the combination's efficacy remains lower than permethrin's, indicating a need for formulation optimization, such as nanoemulsions, to enhance bioavailability and stability, as suggested by Pavoni *et al.* [41]. Moreover, the *In silico* results highlight thymol and γ-muurolene as key bioactive compounds, but their variable PK/PD profiles suggest tailored applications (e.g., thymol for systemic toxicity, γ-muurolene for contact/volatile effects). Future studies should explore additional protein targets

and validate docking results through *in vitro* enzyme inhibition assays. The higher variability in *OgEO*'s pupicidal activity (LC_{50} = 9.7 ± 4.0 µg/mL) warrants further investigation to optimize its consistency, possibly through standardized extraction methods.

Conclusion

This study demonstrates that permethrin exhibits superior insecticidal efficacy against *Anopheles gambiae* across all life stages compared with *Ocimum gratissimum* (*OgEO*) and *Cymbopogon citratus* (*CcEO*) essential oils and their combination, with significantly lower LC_{50} values ($p < 0.05$). The *OgEO+CcEO* combination, however, shows promising synergistic effects, particularly in larvicidal (LC_{50} = 1.6 ± 0.3 µg/mL) and ovicidal (LC_{50} = 1.3 ± 0.2 µg/mL) activities, outperforming individual oils. *In silico* analyses reveal that thymol (from *OgEO*) and γ-muurolene (from *CcEO*) strongly bind to mosquito proteins like catalase and chorion peroxidase, supporting their insecticidal mechanisms.

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