

Original Research Article

Mosquito repellent and anti-ageing potential of *Ocimum gratissimum* essential oil

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Abstract

Purpose: To assess *Ocimum gratissimum* essential oil (OgEO) repellent and anti-ageing potentials for possible cosmeceutical application.

Methods: *Ocimum gratissimum* essential oil was extracted from *O. gratissimum* leaves by hydro-distillation and analyzed via GC-MS. Repellent activity was tested against *Anopheles gambiae* using the mosquito landing assay. Anti-ageing effects were assessed by *in vitro* tyrosinase and elastase inhibition assays, with kinetic analysis via Lineweaver-Burk plots and cytotoxicity by the MTT assay. *In silico* docking targeted *A. gambiae* AgamOBP1 and P5CR for repellency, and tyrosinase/elastase for anti-ageing, with ADMET profiling of key compounds.

Results: The extracted oil repelled *A. gambiae* ($RD_{50} = 37.4 \mu\text{g/mL}$) when compared to *N, N*-diethyl-meta-toluamide (DEET; $23.7 \mu\text{g/mL}$). It inhibited tyrosinase ($IC_{50} = 18.70 \mu\text{g/mL}$; kojic acid: $5.33 \mu\text{g/mL}$) competitively and elastase ($IC_{50} = 9.35 \mu\text{g/mL}$; ursolic acid: $6.66 \mu\text{g/mL}$) non-competitively. *In silico* analysis identified dihydrogeraniol and phytol as bioactive compounds with strong binding to repellent and anti-ageing targets, showing favourable ADMET properties.

Conclusion: The essential oil exhibits potent mosquito repellent and anti-ageing properties, with dihydrogeraniol and phytol as key actives, deserving further investigation as natural cosmeceutical alternatives.

Keywords: Mosquito, Ageing, *Ocimum gratissimum*, Essential oil, Tyrosinase, Elastase

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INTRODUCTION

Mosquitoes are a critical global health concern. They are responsible for transmitting several diseases, including malaria [1]. In sub-Saharan Africa (SSA), the *Anopheles gambiae* is a primary vector for *Plasmodium falciparum*, the protozoan responsible for most malaria cases. Malaria remains a pressing public health challenge in SSA, with the World Health Organization reporting 263 million cases and 597,000 deaths worldwide in 2023. Of these, 94

% of cases (246 million) and 95 % of deaths (569,000) occurred in SSA, with Nigeria accounting for 25.9 % of cases and 30.9 % of deaths [1].

Efforts to curb malaria rely heavily on vector control strategies, including insecticide-treated nets (ITNs), indoor residual spraying (IRS), and synthetic repellents. ITN usage has declined in some regions due to discomfort from reduced indoor ventilation and skin irritation [2]. Mosquito resistance to common IRS, such as pyrethroids

and organophosphates, impairs the efficacy of IRS [3]. N, N-diethyl-meta-toluamide (DEET), a synthetic repellent, while widely used, causes skin irritation and inhibits acetylcholinesterase, posing health risks [4]. These challenges highlight the urgent need for innovative, safer, and more sustainable mosquito control approaches to reduce disease transmission.

In many African communities, traditional practices utilize aromatic herbs to repel mosquitoes [5]. Research has identified plant volatile compounds (PVCs), such as linalool and indole from these aromatic herbs, as effective in disrupting mosquito host-seeking behaviour, offering a promising avenue for developing novel repellents [5].

The essential oil of *Ocimum gratissimum*, one of such aromatic herbs, has shown potent repellent activity against *An. gambiae* [4]. However, beyond repellency, plant-derived essential oils are gaining attention in cosmeceuticals for their ability to modulate enzymes like tyrosinase and elastase, which are involved in skin ageing, suggesting potential dual applications [6]. Developing the essential oil of *O. gratissimum* could address both public health and cosmetic needs.

This study, therefore, investigated the *An. gambiae* repellent and skin anti-ageing potentials of *O. gratissimum* essential oil using experimental and in silico approaches, with the hope of identifying the active compounds for possible cosmeceutical development as active ingredients in all-in-one biorepellent and skincare products.

MATERIALS AND METHODS

Plant Harvest and Authentication

The plant, *Ocimum gratissimum* (Scent leaf), was harvested fresh from their natural habitats in Abraka (latitudes 5° 45' and 5° 50' North of the Equator and longitudes 6° 06' and 6° 15' East of the Greenwich Meridian), Ethiope East LGA of Delta State, Nigeria. It was identified using the LeafSnap App at the point of collection, but was thereafter authenticated (DELSUH 196) by a taxonomist, Mr. O.E. Michael, in the Department of Botany, Delta State University (DELSU), Abraka.

Pulverization of Harvested Plant Leaves

The leaves of the authenticated plant were plucked, air-dried for three weeks and then

ground into fine powder using a mortar and pestle.

Extraction of Essential Oil from the Powdered Sample

The *O. gratissimum* essential oil (OgEO) was extracted using the hydro-distillation method as described by Begum *et al.* [7] using the original Clevenger-type apparatus (Mayalab Instruments, India). The extracted oil was stored refrigerated (4 °C) for use.

Organoleptic Tests for OgEO

The essential oil's smell and appearance were evaluated by randomly selected 10 panelists as previously described [8].

Determination of OgEO's Refractive Index

The refractive index of the essential oil was evaluated using an Abbe refractometer (A. KRUSS Optronic GmbH-DR6300- TF, Hamburg, USA) at 25 °C [5].

GC-MS Analysis of Extracted Oil

The compounds in the extracted OgEO were identified using the GC-MS trimethyl silyl derivatization (TMS) method [9].

Computational Study of Mosquito Repellency

Ligand preparation: An SDF ligand file containing the GC-MS identified compounds was imported into the Maestro workspace and was prepared using the virtual screening workflow panel.

Protein preparation: The repellent protein, *Anopheles gambiae* odour binding protein 1 (AgamOBP1), and pyrroline-5-carboxylate reductase (P5CR), flight energizer mosquito (*An. gambiae*) protein were downloaded from the protein data bank and saved in PDB file format. The proteins were then opened in the Maestro workspace and were prepared in the protein preparation workflow by removing water molecules, side chain atoms and missing hydrogen adjustment.

Receptor grid generation: The binding site for AgamOBP1 and P5CR was defined using coordinates from their structural data.

Docking: The docking was performed with the prepared ligands and proteins using the generated grid file.

Repellent Assays

Evaluation of *in vitro* repellent property of OgEO using mosquito landing technique:

Mosquitoes were reared from larvae collected in stagnant water and identified based on their morphological features. They were sustained on a 10 % sucrose solution for four days under ambient room conditions, with a 12-hour light/12-hour dark cycle, prior to experimentation [10]. Adult mosquitoes were used after an aging period of 3–5 days. For the assay, a sheet of paper was marked into 10 × 10 cm grids, and each grid was treated with varying concentrations of essential oil (0.1, 0.5, 1, 2 and 5%). A group of 20 – 30 female *Anopheles gambiae* mosquitoes was then released into a cage containing the treated grids, and mosquito landings on each square were recorded over 10 – 15 minutes. Percentage repellency (R) was calculated using Eq 1.

$$R (\%) = (N_1 - N_2 / N_2) 100 \dots\dots\dots (1)$$

Where N_1 is the number of mosquitoes landing on control square, and N_2 is the number of mosquitoes landing on treated square.

Cytotoxicity assay

The cytotoxicity of OgEO was assessed using an MTT assay on Vero cells cultured in DMEM with 10 % fetal bovine serum, 1% penicillin-streptomycin, and 1 % L-glutamine at 37 °C in 5 % CO₂ [11]. Cells were seeded at 5 × 10⁴ cells/well in 96-well plates and incubated for 24 hours. OgEO was diluted in DMEM (0.01–1000 µL, < 0.5 % DMSO). After 24 hours of exposure, 20 µL of 5 mg/mL MTT solution was added, followed by 4 hours of incubation. Formazan crystals were dissolved in acidified isopropanol (4 mM HCl, 0.1% NP40), and absorbance was measured at 590 nm. Cytotoxicity was expressed as a percentage of the control, with IC₅₀ determined via dose-response curves. The selectivity index was calculated using Eq 2.

$$\text{Selectivity index (SI)} = CC_{50} / IC_{50} \dots\dots\dots (2)$$

In vitro skin anti-ageing assays

Tyrosinase inhibition activity

This was determined as described by Mansour *et al* [12] with L-3,4-dihydroxyphenylalanine (L-DOPA) substrate. The OgEO was dissolved in DMSO and further diluted in potassium phosphate buffer (50 mM, pH 6.5). Assays were carried out in a 96-well plate and absorbance was read on a Multiskan FC microplate reader

(Thermo Scientific Technologies, Shanghai, China). The oil (70 µL) was mixed with 30 µL of tyrosinase (333 U/mL in phosphate buffer, pH 6.5). After 5 min of incubation at room temperature, 110 µL of substrate (12 mM L-DOPA) was added and the reaction mixture was further incubated for 30 min. Kojic acid was used as a positive control, and a blank contained all the components except L-DOPA. Absorbance was measured at 492 nm, and tyrosinase inhibition (T) percent was calculated as shown in Eq 3.

$$T (\%) = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) 100 \dots\dots\dots (3)$$

where A_{control} and A_{sample} are the absorbance values of the positive control and test reaction mixture, containing kojic acid and the oil, respectively. The IC₅₀ values were determined from linear regression.

Determination of tyrosinase inhibition kinetics

Kinetic parameters (K_m and V_{max}) were derived from Lineweaver–Burk plots using L-DOPA concentrations (100, 200, 250, 500 µg/mL) and OgEO at its IC₅₀.

Elastase inhibitory activity

Elastase inhibition was assessed using N-succinyl-(Ala)3-nitroanilide as a substrate [13]. OgEO was mixed with the substrate in Tris-HCl buffer (0.1 M, pH 8.0), followed by elastase addition. Absorbance was measured at 410 nm. Inhibition was calculated using Eq 4.

$$\text{Ellnh} (\%) = (A_o - A_s / A_o) 100 \dots\dots\dots (4)$$

where A_o is the absorbance of the negative control (solution where, instead of oil, the Tris-HCl buffer was used) and A_s is the absorbance of the *O. gratissimum* oil. Ursolic acid served as the standard elastase inhibitor.

In silico study

Tyrosinase and elastase structures were retrieved from AlphaFold and UniProt, prepared using Glide's protein preparation wizard, and optimized via energy minimization. Ligand preparation, binding site prediction, and virtual screening were performed as described [14].

Statistical analysis

All data were presented as means ± standard error of the mean of triplicate measurements. Statistical significance was set at $p < 0.05$,

analyzed using one-way ANOVA and Tukey post hoc test using GraphPad Prism version 10.4.0: Oct. 2024 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

The results obtained for percentage yield and organoleptic characteristics of essential oil from *Ocimum gratissimum* are presented in Table 1.

GC-MS profile of OgEO

The chromatogram of compounds obtained from the GC-MS analysis of OgEO is displayed in Figure 1, while Table 2 shows the identified compounds.

The identified compounds are combinations of both natural and semi-synthetic phytochemical analogues, having various biological functions, and belonging to different phytochemical classes (alcohols and polyols, ketones and oxygenated terpenes, polyphenols, alkanes and hydrocarbons, terpenes, and carboxylic acids).

Table 1: Percentage yield, refractive index, and organoleptic characteristics of the extracted essential oil from *Ocimum gratissimum*

Parameter	Observation
<i>O. gratissimum</i> powder (g)	700
Oil obtained from <i>O. gratissimum</i> (mL)	9.00
Yield of oil (%)	1.29
Refractive index of oil	1.32
Colour of oil	Green
Smell of oil	Mildly offensive

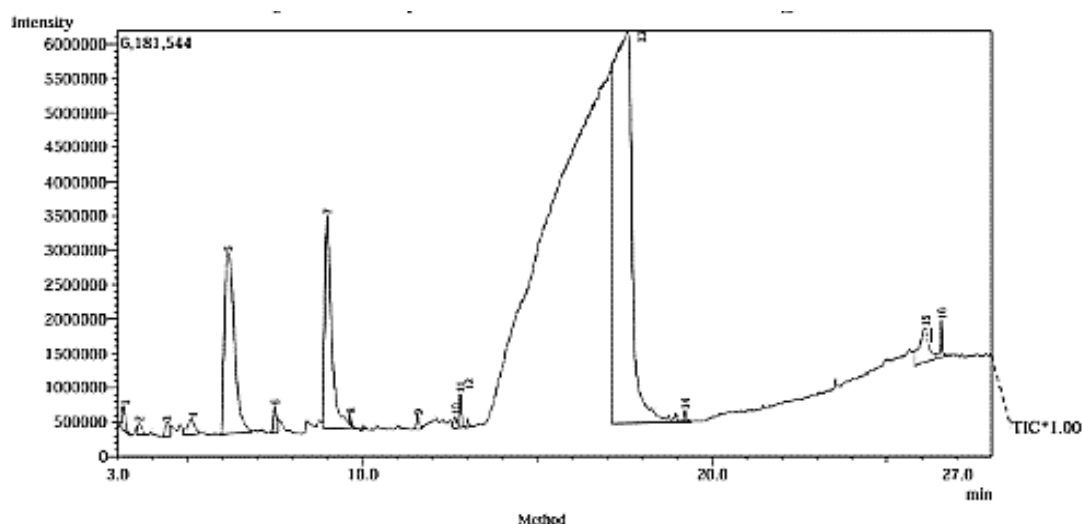


Figure 1: GC-MS analysis of *Ocimum gratissimum* essential oil (OgEO)

Table 2: The GC-MS identified compounds present in OgEO

Compound	MF	MW	RT	PA
1,3-Dipropylene glycol	C ₆ H ₁₄ O ₃	134	3.17	0.62
Cyclohexanone	C ₆ H ₁₀ O	98	3.61	0.46
Phenol	C ₆ H ₆ O	94	4.42	0.58
2-Methyloctane	C ₉ H ₂₀	128	5.12	0.97
Acetic acid	C ₇ H ₁₄ O ₂	130	6.16	15.65
Phenoxyethene	C ₈ H ₈ O	120	7.50	0.75
Resorcinol	C ₆ H ₆ O ₂	110	8.99	12.44
1,2-Dimethyl-3-nitro-4-nitroso-benzene	C ₈ H ₈ N ₂ O ₃	180	11.59	0.33
Bicyclo(4.1.0)heptan-3-ol	C ₁₀ H ₁₈ O	154	12.59	0.26
1-(7-Hydroxy-1,6,6-trimethyl-10-oxatricyclo(5.2.1.0(2,4)dec-9-yl)ethenone	C ₁₄ H ₂₂ O ₃	238	12.74	0.61
3-Acetyl octadien-2-one	C ₁₀ H ₁₄ O ₂	166	12.95	0.13
3-O-Methyl-D-glucose	C ₇ H ₁₄ O ₆	194	17.13	63.13
Phytol	C ₂₀ H ₄₀ O	296	19.10	0.19
Dihydrogeraniol	C ₁₀ H ₂₀ O	156	19.10	0.14
Heneicosane	C ₂₁ H ₄₄	296	26.43	3.02
Squalene	C ₃₀ H ₅₀	410	26.75	0.70

MF= Molecular formular, MW= Molecular weight (g/mol), RT= Retention time (min), PA= Peak area (%)

***In silico* study of *Anopheles gambiae* repellent properties of OgEO**

Docking profile

The values obtained from the Autodock Vena analysis of the sixteen (16) compounds identified in the GC-MS profile against the *Anopheles gambiae* mosquito odour binding protein 1 (AgamOBP1) are displayed in Table 3.

The docking data (Table 3) predicted phytol as the topmost bioactive compound, outperforming the standard.

The post-docking diagrams showing the interaction of the two compounds with AgamOBP1 compared with the standard are shown in Figures 2 a - c.

Figure 2 a show the binding interaction of dihydrogeraniol with AgamOBP1, indicating that dihydrogeraniol formed hydrophobic bond with Met 91, Met 89, Ala 88, Trp 114, Tyr 122, Phe

123, Met 84, Leu 80, Leu 76, Leu 96 and glycine bond with Gly 92. These binding interactions contributed to the overall stability of the complex. Leu 96, Met 91, Met 89, Ala 88, Met 84, Phe 123, Tyr 122, Val 64, Ala 62, Leu 19, Leu 15, Phe 59, Leu 58, Leu 80, Trp 114, Leu 76, Leu 73, and positive charge bond with Lys 93, and glycine bond with Gly 92 are all examples of binding interaction of phytol with AgamOBP1 are depicted in Figure 2c. These interactions demonstrate that picaridin produced positive charges (Lys 93), and hydrophobic bonds (Leu 96, Met 91, Met 89, Ala 88, Trp 114, Leu 80, Leu 76, Leu 73).

These various interactions contribute to the overall stability of the complexes, suggesting that phytol and dihydrogeraniol are strong inhibitors of AgamOBP1, and this provides computational evidence of *An. gambiae* mosquito repellent property. Furthermore, another target for the study of mosquito repellence is the flight inhibitor target pyrrolines-5-carboxylate reductase (P5CR).

Table 3: Docking profile of dihydrogeraniol and phytol with AgamOBP1

Docking profile	Dihydrogeraniol	Phytol	Picaridin (Standard repellent)
Compound ID	13665263	5280435	125098
Dock score (kCal/mol)	-5.94	-7.31	-5.04
XP Gscore (kCal/mol)	-5.98	-7.31	-5.04
MMGBSA dG Bind (kcal/mol)	-33.01	-66.37	-37.33

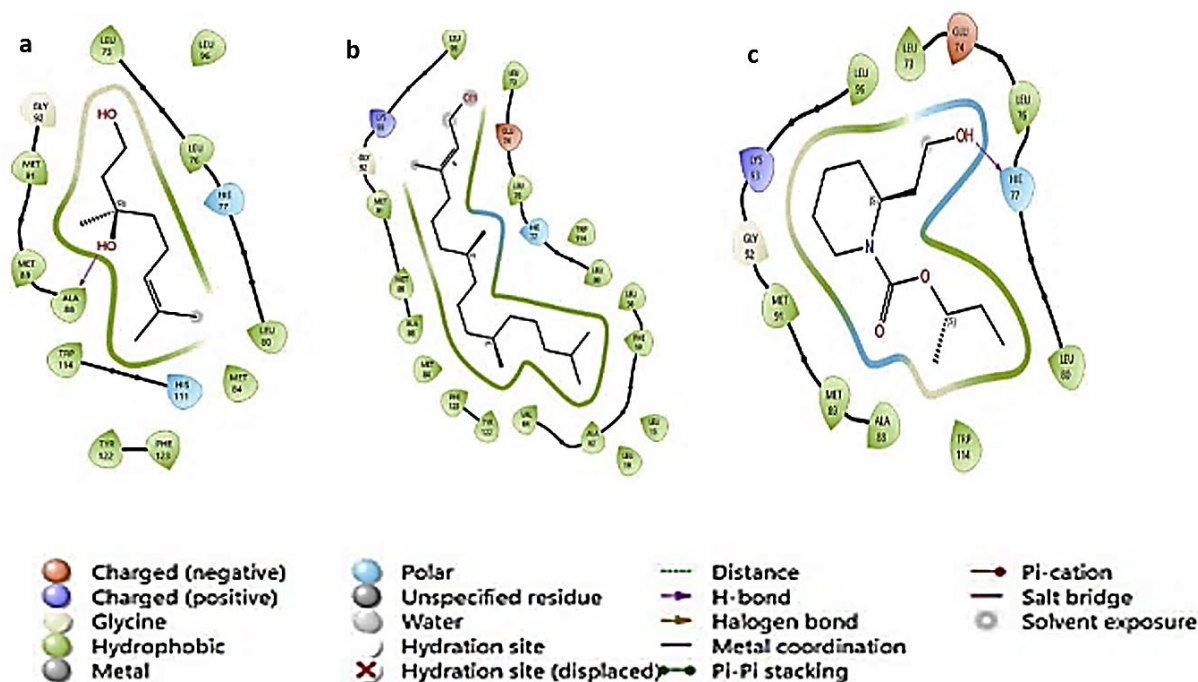


Figure 2: 2D interactions of compounds with *Anopheles gambiae* odour binding protein-1 (AgamOBP1): a. Dihydrogeraniol in complex with AgamOBP1, b. Phytol in complex with AgamOBP1, and c. Picaridin in complex with AgamOBP1

The docking profile of P5CR with phytol, dihydrogeraniol, and oxoproline (standard inhibitor) is shown in Table 4. Then, the residue interaction complexes are shown in Figures 3 a - c.

Dihydrogeraniol, with docking score of -4.94 kCal/mol and MMGBSA dG Bind value of -31.21 kCal/mol, has the strongest binding affinity with pyrroline-5-carboxylate reductase. Phytol, with dock score of -3.79 kCal/mol, had a binding affinity stronger than 4-oxoproline, the standard inhibitor (dock score -2.77 kCal/mol and MMGBSA dG Bind -16.90 kCal/mol).

Figure 3 indicates the binding interaction complexes between dihydrogeraniol, phytol, and 4-oxoproline (standard inhibitor) and the flight inhibitor target (pyrroline-5-carboxylate reductase).

Residue interactions (Figure 3) show that dihydrogeraniol formed two hydrogen bonds with Pro 72, Ser 103, while phytol formed one hydrogen bond with Gln 269, but 4-oxoproline formed two polar hydrogen bonds with Thr 100 and Gln 269.

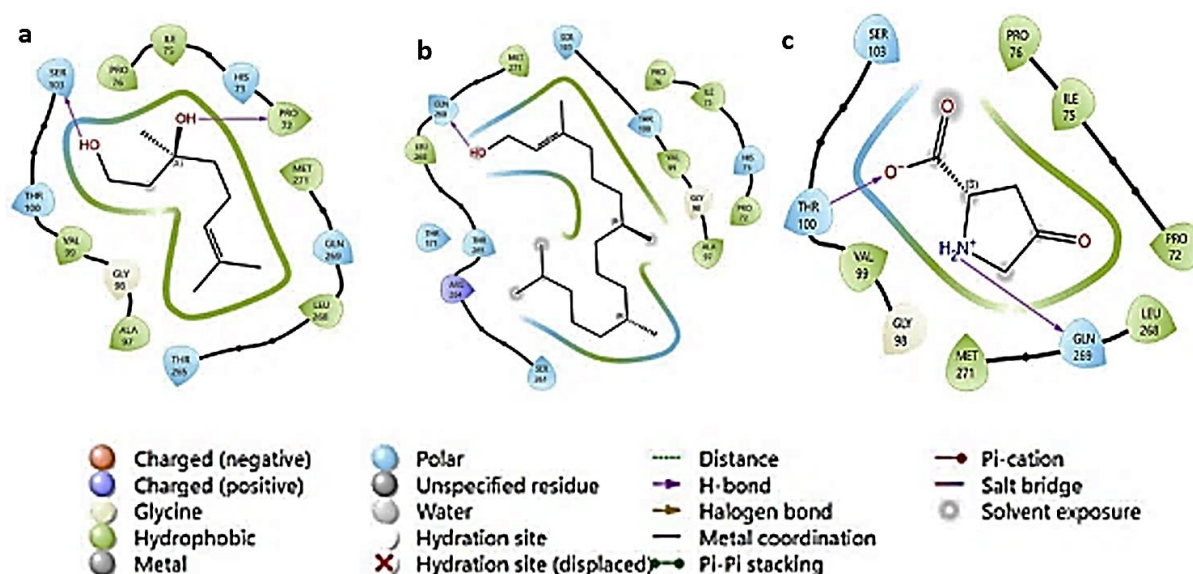
Put together, the *in silico* study on mosquito odour binding and flight inhibition suggests that both phytol and dihydrogeraniol are strong inhibitors of these targets, implying that these compounds of OgEO possess significant mosquito repellent and flight inhibition potentials. *In vitro* preliminary screening of the repellent property of OgEO was then undertaken using the mosquito landing assay.

Evaluation of *in vitro* repellent property of OgEO using mosquito landing technique

The results obtained indicated that the repellent dose of OgEO and DEET at 50 % repellency (RD₅₀) were 37.4 and 23.7 µg/mL, respectively. The values indicate that OgEO is active in repelling *Anopheles gambiae* mosquitoes, which compares well with the standard, DEET ($p > 0.05$). Having affirmed the repellent property of OgEO, using *in vitro* landing assay and predicting the bioactive compounds using *in silico* approach, it became imperative to assess the anti-ageing property of OgEO since it is planned for dermal application.

Table 4: Binding interaction of dihydrogeraniol and phytol with mosquito flight inhibitor target (Pyrroline-5-carboxylate reductase)

Docking profile	Dihydrogeraniol	Phytol	4-Oxoproline (standard inhibitor)
Compound ID	13665263	5280435	107541
Dock-score (kCal/mol)	-4.94	-3.70	-2.70
XP Gscore (kCal/mol)	-4.94	-3.70	-2.83
MMGBSA dG Gind (kcal/mol)	-31.21	-40.50	-16.90



Skin anti-ageing properties of OgEO

The research study further evaluated the biological activities of OgEO in relation to cytotoxicity and inhibitory effects on tyrosinase and elastase, two key enzymes involved in skin ageing, and the results obtained are given in Table 5.

OgEO potential as an enzyme inhibitor was greatly enhanced by the findings of its *in vitro* inhibition and kinetic investigations against the tyrosinase and elastase enzymes (Table 5), which showed good comparisons with the conventional inhibitors (ursolic acid for elastase and kojic acid for tyrosinase). The IC_{50} values indicate that OgEO exhibited moderate inhibitory activity against tyrosinase ($IC_{50} = 18.70 \mu\text{g/mL}$) and stronger activity against elastase ($IC_{50} = 9.35 \mu\text{g/mL}$), when compared with the standard inhibitors, kojic acid ($IC_{50} = 5.33 \mu\text{g/mL}$ for tyrosinase) and ursolic acid ($IC_{50} = 6.66 \mu\text{g/mL}$ for elastase). Although OgEO was less potent than the standard inhibitors, its selectivity index (SI) values of 24.01 for tyrosinase and 48.02 for

elastase suggest a favourable safety profile, particularly for elastase inhibition, indicating low cytotoxicity relative to its inhibitory efficacy.

Kinetic studies reveal OgEO's inhibition mechanisms. For tyrosinase, OgEO raised K_m (142 to 250 $\mu\text{g/mL}$) without changing V_{max} (278 $\mu\text{g/mL/min}$), indicating competitive inhibition, likely due to structural similarities with the substrate. For elastase, OgEO lowered V_{max} (537 to 333 $\mu\text{g/mL/min}$) while K_m remained 222 $\mu\text{g/mL}$, suggesting non-competitive inhibition, unlike ursolic acid. Lineweaver-Burk plots confirm this unique mechanism. Virtual assessment of repellent compounds (dihydrogeraniol, phytol) is detailed in Table 6 (docking profile) and Figure 5 (residue interactions).

Dihydrogeraniol docked to the active site of human tyrosinase, and had the highest binding affinity with docking score and XP GScore of -4.06 kCal/mol, followed by phytol with -3.11 kCal/mol.

Table 5: *In vitro* inhibition and kinetic data for OgEO interaction with tyrosinase and elastase

Parameter	Tyrosinase	Elastase
<i>In vitro</i> inhibition		
IC_{50} ($\mu\text{g/mL}$)		
OgEO	18.70	9.35
Standard inhibitors	5.33	6.66
Cytotoxicity of OgEO, $CC_{50} = 449 \mu\text{g/mL}$		
Selectivity Index (CC_{50}/IC_{50}) of OgEO	24.01	48.02
Kinetic studies		
K_m ($\mu\text{g/mL}$)	142	222
K_m ($\mu\text{g/mL}$) in the presence of OgEO	250	222
K_m ($\mu\text{g/mL}$) in the presence of standard inhibitor	418	222
V_{max} ($\mu\text{g/mL/min}$)	278	537
V_{max} ($\mu\text{g/mL/min}$) in the presence of OgEO (inhibitor)	278	333
V_{max} ($\mu\text{g/mL/min}$) in the presence of standard inhibitor	278	305
Inhibition type	Competitive	Non-competitive

Standard inhibitor of tyrosinase = kojic acid, standard inhibitor of elastase = ursolic acid. Kinetic constants (K_m = Michaelis constant, V_{max} = Maximum velocity). Data were obtained from dose-response curves and kinetic plots (Lineweaver-Burk) generated from the average of triplicate determinations. Kinetic assays of inhibitions by OgEO, kojic acid and ursolic acid were conducted in the presence of their IC_{50} values as the inhibitor concentrations

Table 6: Molecular docking profiles of dihydrogeraniol and phytol against tyrosinase and elastase

Docking profile	Dihydrogeraniol	Phytol	Kojic acid	Ursolic acid
Compound ID	13665263	5280435	3840	64945
Tyrosinase				
Dock-score (kCal/mol)	-4.06	-3.11	-6.86	
XP Gscore (kCal/mol)	-4.06	-3.11	-7.77	
MMGBSA dG Bind (kCal/mol)	27.24	19.96	46.66	
Elastase				
Dock-score (kCal/mol)	-4.53	-3.53		-2.96
XP Gscore (kCal/mol)	-4.53	-3.53		-2.96
MMGBSA dG Bind (kCal/mol)	-25.46	-58.04		-38.07

The standard, kojic acid, had a lower dock-score of -6.86 kCal/mol, suggesting it has better binding affinity and a better inhibitor for human tyrosinase protein than the other compounds (dihydrogeraniol and phytol) that still showed significant binding (Table 6).

Dihydrogeraniol, with dock-score and XP GScore of -4.53 kCal/mol, MMGBSA dG, -25.46 kCal/mol, had the highest binding affinity, followed by phytol with -3.53 kCal/mol dock score and XP GScore with -58.04 kCal/mol MMGBSA, both indicating good binding affinity and very

stable complexes with elastase. However, the standard, uric acid, had the least binding affinity (dock value and XP Gscore of -2.96 kCal/mol). Figure 4 shows the residue interaction complexes for tyrosinase with dihydrogeraniol (a), phytol (b), and kojic acid (c), and elastase with dihydrogeraniol (d), phytol (e), and ursolic acid (f). Since the docking scores and residue interactions indicate that dihydrogeraniol and phytol are promising repellent and anti-ageing compounds. The drug-likeness, ADMET, and physicochemical properties were computationally evaluated, with results shown in Table 7.

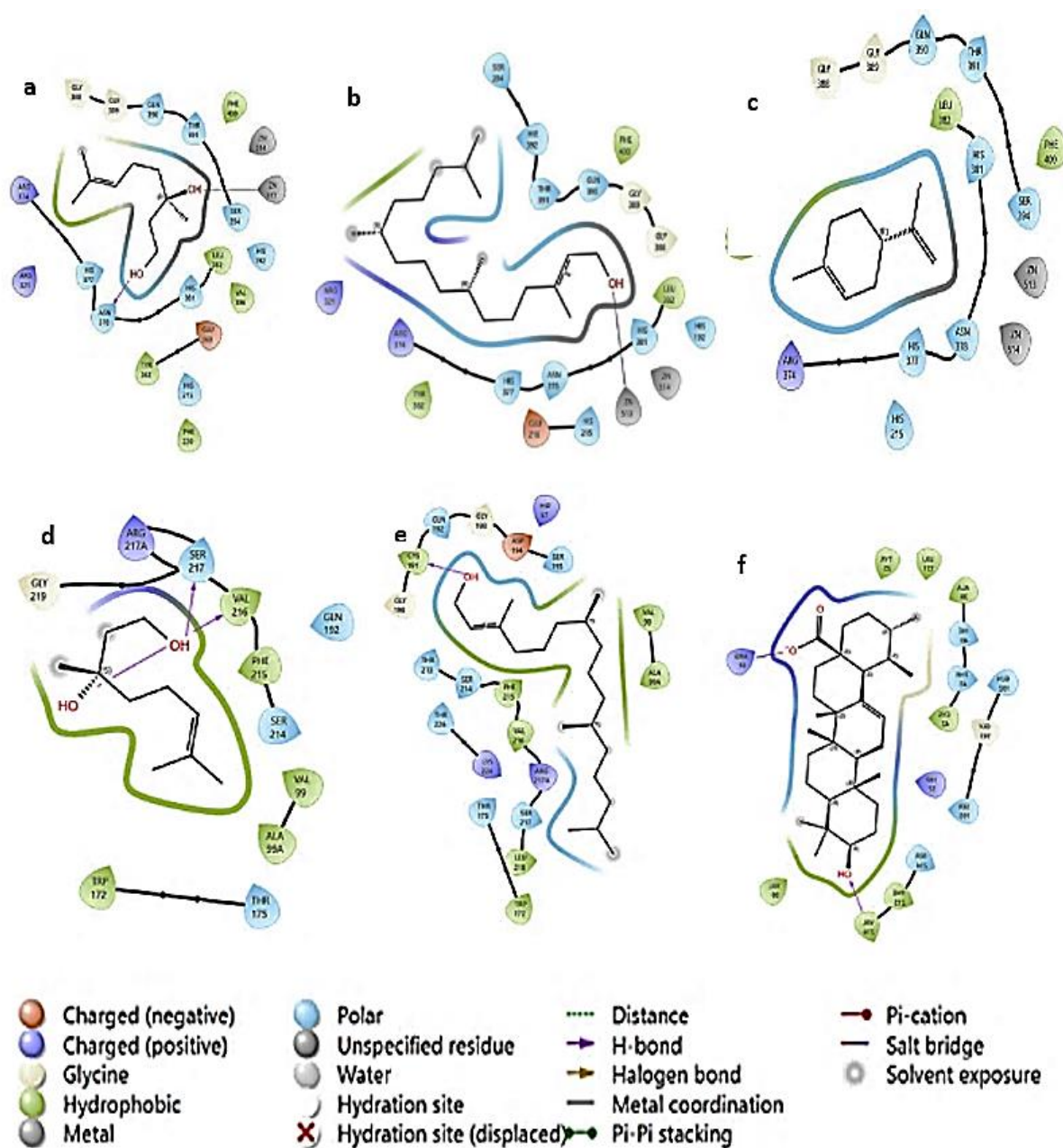


Figure 4: Binding complexes of tyrosinase with dihydrogeraniol (a), phytol (b), and kojic acid (c) and elastase binding complexes with dihydrogeraniol (d), phytol (e), and ursolic acid (f).

Table 7: Drug likeness, physicochemical and ADMET Properties of dihydrogeraniol and phytol obtained from OgEO

Property	Dihydrogeraniol	Phytol
Drug likeness		
Ionization constant (p^{ka}) (Basic)	7.60	7.22
Partition coefficient ($\log P$)	2.21	8.01
Distribution ($\log D$)	0.67	5.44
Solubility ($\log S$)	-1.38	-5.47
Physicochemical		
Molecular weight (g/mol)	172.26	296.53
Molecular formula	$C_{10}H_{18}O$	$C_{20}H_{40}O$
TPSA	40.46 Å ²	20.23 Å ²
HBA	2	1
HBD	2	1
NRB	5	13
Pharmacokinetic (ADMET)		
Absorption		
Caco2 permeability (cm/s)	1.573	1.399
Intestinal absorption (human)	92.899	90.643
Skin permeability	-2.883	-2.631
P-glycoprotein substrate	No	No
P-glycoprotein I inhibitor	No	No
P-glycoprotein II inhibitor	No	No
Distribution		
VDss (Human)	-0.139	0.385
Fraction unbound (Human)	0.53	0
BBB permeability	0.229	0.793
CNS permeability	-2.644	-1.527
Metabolism		
CYP2D6 substrate	No	No
CYP3A4 substrate	No	Yes
CYP1A2 inhibitor	No	Yes
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	No
Excretion/Toxicity		
Total Clearance	1.517	1.686
Renal OCT2 substrate	No	No
AMES toxicity	No	No
Max. tolerated dose (human)	1.197	-0.301
hERG I inhibitor	No	No
hERG II inhibitor	No	Yes
Oral rat acute toxicity (LD ₅₀ ; mol/kg)	1.564	1.848
Oral rat chronic toxicity (LOAEL)	2.785	1.232
Hepatotoxicity	No	No
Skin sensitisation	Yes	Yes
<i>T.pyriformis</i> toxicity (µg/L)	-0.212	1.714
Minnow toxicity (mM)	1.493	-1.137

Dihydrogeraniol is more basic (higher p^{ka}) and water-soluble ($\log S$) than phytol, which is more lipophilic (higher $\log P$). Phytol is heavier (296.53 g/mol) than dihydrogeraniol (136.23 g/mol). Hydrogen bond donors/acceptors vary, impacting solubility and interactions. Dihydrogeraniol has higher intestinal absorption (92.9%) than phytol; both have low skin permeability. Phytol distributes better in tissues (positive VDss), while dihydrogeraniol has more free drug in blood. Phytol crosses BBB better but has low CNS penetration. It's a CYP3A4 substrate and CYP1A2 inhibitor. Phytol clears faster (1.686 vs. 1.517). Neither is mutagenic (AMES). Dihydrogeraniol has a higher tolerated dose (1.197 vs. -0.301) and LOAEL (2.785), indicating better chronic safety

DISCUSSION

Plant-derived essential oils have emerged as promising candidates for managing malaria vectors due to their eco-friendlier nature and efficacy. Research has shown that essential oils from *Pinus* spp., *Cymbopogon* spp. (citronella), and *Ocimum* spp. exhibit strong repellent activity

against various *Anopheles* species, with protection times ranging from 5 to 11.5 hours depending on the plant and mosquito species [4]. Specifically, essential oil from *Ocimum gratissimum* (OgEO) demonstrated a repellent time length (RTL) of 60 ± 17 minutes against *Anopheles gambiae* (*sensu stricto*), comparable

with DEET's performance, making it a potential natural alternative [5].

In this study, the yield, colour and physicochemical properties of the extracted essential oil from *O. gratissimum* are consistent with earlier reports [4,5], indicating good yield and acceptance profile. For the mosquito landing assays, OgEO exhibited significant repellency ($RD_{50} = 37.4 \mu\text{g/mL}$), closely matching DEET ($RD_{50} = 23.7 \mu\text{g/mL}$) with no statistical difference ($p > 0.05$), supporting its potential as a viable substitute for synthetic repellents.

Gas chromatography-mass spectrometry (GC-MS) analysis of OgEO by Osei-Owusu *et al.* [5], identified key bioactive compounds, including thymol, citral, geraniol, and β -caryophyllene, known for their insect-repellent and insecticidal effects. For instance, citral and geraniol disrupt host-seeking behaviour in *Aedes albopictus* by interfering with olfactory perception [15]. In this study, *in silico* prediction further revealed that among the GC-MS identified 16 compounds of OgEO, dihydrogeraniol and phytol potentially inhibited *Anopheles gambiae* mosquito odorant-binding protein 1 (*AgamOBP1*) and pyrroline-5-carboxylate reductase (*AgamP5CR*). *AgamOBP1* facilitates mosquito detection of host odours like indole and ammonia, critical for blood-feeding [16]. *AgamP5CR* supports proline biosynthesis, contributing to flight energy during host-seeking [17]. These compounds outperformed standard inhibitors in molecular docking, suggesting a novel mechanism for vector control.

Beyond repellency, OgEO demonstrated significant inhibition of human tyrosinase and elastase, enzymes implicated in skin ageing, with efficacy comparable with standard inhibitors, kojic acid (tyrosinase) and ursolic acid (elastase). Kinetic analyses indicated competitive inhibition of tyrosinase and non-competitive inhibition of elastase, while *in silico* studies confirmed strong binding of dihydrogeraniol and phytol to these enzymes. These findings align with prior research, where 17 of 100 plant extracts showed >30% inhibition of tyrosinase and elastase at 50 $\mu\text{g/mL}$ [6]. Tyrosinase, a key enzyme in melanin biosynthesis, contributes to hyperpigmentation, while elastase degrades elastin and collagen, leading to skin sagging and wrinkles [18]. By inhibiting these enzymes, OgEO offers potential cosmeceutical applications, reducing skin ageing signs, while also, decreasing mosquito attraction through lower melanin production [19].

The dual functionality of OgEO as a mosquito repellent and skin anti-ageing agent is driven by its bioactive compounds, which exhibit

favourable pharmacokinetic and ADMET profiles, supporting their drug-like potential.

CONCLUSION

OgEO's potent mosquito-repellent and skin anti-ageing properties, propelled by compounds like dihydrogeraniol and phytol, position it as a promising natural alternative to synthetic repellents and a candidate for cosmeceutical development. Further research is, however, needed to optimize its formulation and ensure safety for widespread use.

DECLARATIONS

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Ethical approval

Ethical approval (RBC/FBMC/DELSU/24/312) for the study was obtained from the Research and Ethics Committee, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. OI designed

the study, sourced funding support, supervised the experiments, and prepared the manuscript. KGR and AJO conducted the experiments and generated the data, while OOB and DV performed the statistical analyses and interpreted the results. All authors read and approved the manuscript to be considered for journal publication.

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