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Article

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Posted Date: September 2nd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7330436/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on May 29th, 2026. See the published version at <https://doi.org/10.1038/s41598-026-55249-7>.

***In-silico* study of the bioactive compounds of *Blighia sapida* koenig revealed a novel *Plasmodium falciparum* Dihydroorotate dehydrogenase inhibitor for malaria**

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ABSTRACT

Facing drug-resistant malaria, this study evaluated the antimalarial potential of bioactive compounds from *B. sapida* ethanolic leaf extract using *in silico* approach

Gas chromatography-mass spectroscopy (GC-MS) analysis was carried out to identify the compounds present in the extract. Molecular docking and dynamic simulation were done to evaluate interactions and stability between the lead compounds and the target protein, PfDHODH using Autodock vina and Gromacs software respectively.

Twenty-one compounds were identified from GC-MS analysis of the extract. The three predominant identified phytochemicals were D-Fructose 3-O-methyl- (21.02%), Phytol (20.77%) and Hexadecanoic acid, ethyl ester (13.11%). Complementary *in silico* molecular docking studies revealed interactions between the 21 compounds and PfDHODH. Seven compounds exhibited pronounced binding affinities (-17.4Kcal/mol to -7.4 Kcal/mol) when compared to the positive control, chloroquine (-7.2 Kcal/mol). Phytol, Ethyl 9,12,15-octadecatrienoate, Hexadecanoic acid methyl ester, Linoleic acid ethyl ester, and Hexadecanoic acid, ethyl ester demonstrated commendable drug-like characteristics and also showed good ADMET properties. Phytol was identified as the most stable inhibitor (RMSD- 0.176, ROG- 1.92, SASA- 166.9, Hydrogen bond-

2.02), followed by Ethyl 9,12,15-octadecatrienoate (RMSD- 0.191, ROG- 1.92, SASA- 171.1, Hydrogen bond- 0.57).

These findings emphasize the potential antimalarial properties of *B. sapida* and shed light on its bioactive compounds.

Keywords: Antimalaria, drug-likeness, *Blighia sapida*, molecular docking, ADMET, molecular simulation

1. Introduction

Malaria continues to pose a serious threat to global public health initiatives despite several attempts to eradicate this illness. It causes significant death in tropical and subtropical areas, especially in Africa, where pregnant women and children are particularly at risk ¹. Although great and significant strides have been made by the global malaria community in the eradication of this menace ², regrettably, it seems that the number of instances of malaria has increased post-pandemic ³. According to the World Malaria Report ⁴, there were over 249 million cases of malaria worldwide, which is 16 million more than the 233 million instances that were before the pandemic in 2019 suggesting a need to explore more proactive measures for its control.

Numerous antimalarial medications have been developed over the years, however, the efficiency of these medications is limited by the emergence of multidrug resistance, particularly in *P. falciparum*, which is one of the most deadly malaria parasites ^{5,6}. Infact, evidence of emergence of parasite resistance to artemisinin-based combination therapies (ACTs), recommended by the WHO for treating uncomplicated malaria, have also been reported to face resistance in African nations, including Nigeria ^{7,6} which suggest the need to develop novel drug with unique mode of action.

Plasmodium falciparum dihydroorotate dehydrogenase (PfDHODH) is an essential enzyme in the pyrimidine biosynthesis pathway that catalyzes the oxidation of dihydroorotate to orotate, a key step in the de novo synthesis of pyrimidine nucleotides required for DNA and RNA synthesis in

57 *P. falciparum*⁸. Since *Plasmodium* species, including *P. falciparum*, cannot salvage pyrimidines
58 from the host environment, they rely heavily on de novo synthesis for their nucleotides, making
59 PfDHODH an attractive and essential therapeutic target for antimalarial drug development⁹.
60 Unlike the human version of DHODH, which is found in the mitochondria, PfDHODH is located
61 within the apicoplast, an organelle that is vital to the survival of the parasite. The unique location
62 of PfDHODH and its differences from human DHODH have made it an ideal target for selective
63 inhibition, minimizing potential off-target effects on the human host⁹. Brequinar is one of the
64 prominent identified PfDHODH inhibitors¹⁰, and others, such as tipifarnib and IMU-838, have
65 been shown to inhibit parasite growth by preventing the synthesis of pyrimidines, which in turn
66 disrupts DNA replication, a process crucial for the division and proliferation of the parasite¹¹. In
67 recent times, several newly identified compounds have emerged as potential hit candidates, and
68 others are currently undergoing clinical evaluation for malaria treatment^{6,12,13}. Among these,
69 DSM265 identified through experimental studies has progressed through Stage 2 clinical trials⁹

70 The woody, perennial fruit tree *Blighia sapida*, also known as the Ackee or Akee apple and locally
71 called *Isin* in Southwestern Nigeria is a member of the Sapindaceae family. It is common in the
72 woodlands of numerous West African nations including Nigeria^{14,15}. Historically, it is known for
73 its medicinal property, and the different parts of the tree have been used to treat many illnesses,
74 including cancer, childhood fever, gonorrhea, diarrhea, backache, constipation, psychosis, hernia,
75 stomachache, rheumatism, typhoid, and malaria¹⁵. Furthermore, empirical evidences have
76 demonstrated that *Blighia sapida* possesses antimicrobial properties, antidiarrheal, anticancer,
77 antidiabetic, antimalarial and antioxidant properties¹⁶⁻¹⁹. The antimalaria properties of this tree
78 make it a candidate for further studies.

79 *In silico* studies are computational methods that have become an essential component of modern
80 drug discovery, offering rapid and cost-effective means of identifying and evaluating potential
81 therapeutic agents before experimental validation. Among the key techniques, molecular docking
82 predicts interactions between small molecules and biomolecular targets, playing a crucial role in
83 identifying potential drug candidates²⁰. ADMET (absorption, distribution, metabolism, excretion
84 and toxicity) analysis on the other hand, have been instrumental in the preliminary screening and
85 validation of bioactive compounds^{20,21}, while molecular dynamic simulation enables the

exploration of molecular conformations, protein folding, protein-ligand interactions, and macromolecular dynamics that are difficult to capture with experimental techniques²².

However, to the best of our knowledge, there is no report on the in-silico study of *Blighia sapida* bioactive compounds as a candidate antimalarial agent despite its antimalarial potential²³. Therefore, this study seeks to identify the bioactive component of *Blighia sapida* using GC-MS. The molecular docking poses, screening, and conformational dynamics evaluation of the bioactive compounds identified by GC-MS were done using molecular docking, drug-likeness and ADMET profiling, and molecular dynamic simulations. This research is significant in the ability to combat the growing prevalence of antimalarial drug resistance and contributes to advancing more effective and affordable malaria treatments.

2. Methodology

2.1 Gas chromatography-mass spectroscopy analysis of *Blighia sapida*

Ethanol extract of the leaves of *Blighia sapida* was analyzed using an Agilent 8860 Gas Chromatograph equipped with a 5977B Mass Spectrometry Detector (MSD) system, fitted with an Elite-5MS capillary column (30 × 0.25 µm ID × 0.25 µm df) containing 5% diphenyl and 95% dimethyl polysiloxane. GC-MS detection was performed using an electron ionization system in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) served as the carrier gas at a constant flow rate of 1 mL/min, with an injection volume of 1 µL and a split ratio of 10:1.

The injector temperature was set at 300 °C, while the ion-source temperature was maintained at 250 °C. The GC oven temperature was programmed to start at 110 °C (held for 1 minute), then increased at a rate of 15 °C/min to reach 310 °C (held for 2 minutes). Mass spectra were recorded at 70 eV with a scanning interval of 0.5 seconds, capturing fragment ions in the range of 45 to 450 Da. A solvent delay of 0 to 3 minutes was applied.

2.2 Ligand selection and preparation

The 21 phytochemical compounds generated from the GC-MS analysis, including the standard drug, chloroquine, were downloaded from the NCBI PubChem database in SDF format for this

study. Conversion of ligands from SDF to PDF files was done using Pymol tools and was prepared using the Autodock Vina followed by Lipinski's filter²⁴.

2.3 Protein target selection and preparation

Crystal structure of DHODH (PDB: 6I55) complex protein was retrieved from the Protein Data Bank (PDB) (Figure 1). Preparation of the protein, including water removal, addition of polar hydrogen, energy minimization, and determination of the binding site were carried out using Discovery Studio²⁵. The receptor grid, which estimates the area around the active site in terms of coordinates x, y, and z, was then generated (DHODH: 12.368 34.480 24.127). The grid dimensions 40*40*40 were used with a grid spacing of 0.375.

2.4 Molecular Docking

Molecular docking of the 21 ligands obtained from GC-MS analysis with the target protein DHODH was completed using AutoDock tools and Molecular Operating Environment (MOE) with the standard procedure²⁶. Different conformations of the ligands were generated during the docking process, and the final energy refinement of each ligand pose was carried out. The docking score for the optimal pose of each bioactive compound within the target protein was then calculated. The lowest binding energy ligand enzyme complexes were recorded for the target protein. Discovery Studio visualizer was used to visualize the docking complex interactions of the ligand-to-protein complex.

2.4.1 Docking Using AutoDock Vina

Initial molecular docking was carried out using AutoDock Vina 1.5.7²⁷. The protein and ligands were converted into PDBQT format using AutoDock Tools. A grid box was set around the active site, and docking was performed with an exhaustiveness value of 8. Vina provided multiple binding poses ranked by binding affinity (kcal/mol), and the top-ranked pose was used for further analysis.

2.4.2 Docking Using Molecular Operating Environment (MOE)

For further validation, docking was repeated using MOE's Dock module. The binding pocket was identified using the site finder tool, and ligands were docked into the active site using the triangular matcher algorithm. Poses were scored using London dG (delta G) scoring and refined using GBVI/WSA dG²⁸. The best pose for each ligand was selected based on binding energy and interaction with key residues.

2.5 Interaction and Visualization

Docked complexes were visualized using PyMOL and Discovery Studio Visualizer to observe key interactions such as hydrogen bonds and hydrophobic contacts. Binding energy values and interaction patterns were compared to evaluate the binding strength and stability of each ligand with the target protein.

2.6 Drug-likeness and ADMET Profiling of the Isolated Compounds

The SMILES (Simplified Molecular-Input Line-Entry System) representations of each isolated compound were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) for computational analysis. In silico predictions of physicochemical and pharmacokinetic properties, including absorption, distribution, metabolism, and excretion (ADME), were conducted using the SwissADME platform (<http://www.swissadme.ch/index.php>) and the pkCSM web server (<https://biosig.lab.uq.edu.au/pkcsml>). Drug-likeness, based on Lipinski's Rule of Five, was assessed using SwissADME while pkCSM was utilized to evaluate the ADMET properties (Absorption, Distribution, Metabolism, Excretion, and Toxicity) of the lead compounds.

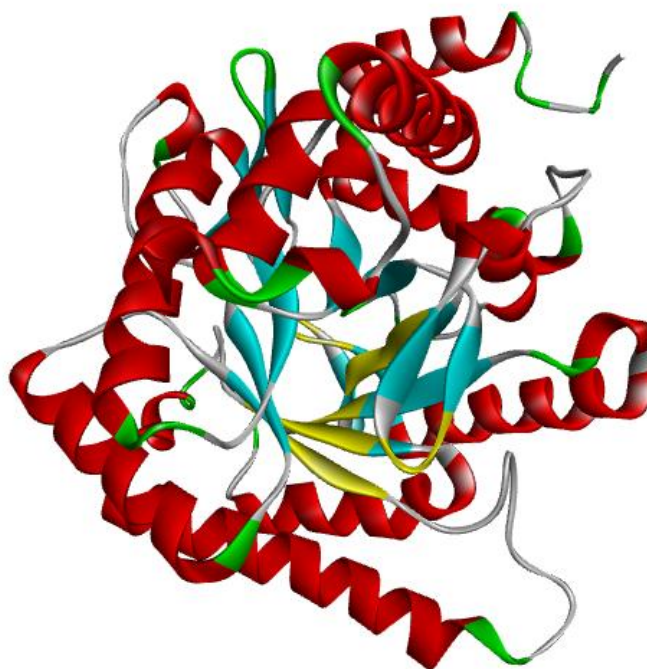


Figure 1: Structure of the *Plasmodium falciparum* dihydroorotate dehydrogenase (pfDHODH) target Protein (Protein DataBank, www.rcsb.org).

2.6.1 Drug-Likeness Assessment

Drug-likeness of selected compounds was evaluated based on key physicochemical descriptors, including molecular weight (MW), lipophilicity (MLOGP), number of hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), and topological polar surface area (TPSA). These

properties were assessed according to Lipinski's rule of five, a widely accepted criterion for predicting oral bioavailability²⁹. Additional drug-likeness parameters such as the number of rotatable bonds and bioavailability score were calculated to complement the primary Lipinski metrics. The bioavailability score, ranging from 0 to 1, estimates the probability that a compound will be orally bioavailable in humans³⁰.

The physicochemical properties were predicted using the SwissADME web tool (<http://www.swissadme.ch/index.php>), which integrates multiple algorithms for *in silico* profiling of small molecules³¹. The platform was employed to calculate MLOGP, TPSA, and other descriptors relevant to oral bioavailability.

2.6.2 ADMET Profiling

The ADMET properties, comprising Absorption, Distribution, Metabolism, Excretion, and Toxicity, were analyzed *in silico* using a combination of predictive models. Key ADMET parameters include absorption, which is assessed via log K_p (skin permeability), human intestinal absorption (HIA), and Caco-2 cell permeability. Distribution: evaluated using blood-brain barrier (BBB) permeability predictions. Metabolism: that focused on the potential interaction with cytochrome P450 (CYP) isoenzymes. Excretion: determined by estimating total clearance and renal excretion likelihood and toxicity which is predicted by using *in silico* toxicity models for hepatotoxicity, AMES mutagenicity, and LD50 (lethal dose 50%). All ADMET parameters were predicted using the pkCSM (<https://biosig.lab.uq.edu.au/pkcsm>) platform, which employs graph-based signatures models for accurate ADMET prediction³². These *computational* tools allowed for rapid, cost-effective screening of candidate compounds, facilitating early-stage assessment of pharmacokinetic behavior and drug safety profiles, consistent with current practices in drug discovery³³.

2.7 Molecular dynamics simulation (MDS)

The methodology in this study utilized Molecular Dynamics (MD) simulations to examine the binding stability, conformational dynamics, and interaction modes between ligands and the target protein. These simulations were conducted using GROMACS version 2021, a widely recognized tool for accurately simulating proteins, lipids, and nucleic acids³⁴. Protein and ligand topologies were generated using the CHARMM36 force fields and further refined using GROMACS and CgenFF protocols^{3,35}. The prepared protein-ligand complex was embedded within a dodecahedron box containing counterions and simple point charge (SPC) water molecules to create a neutral, solvated environment suitable for simulation. To ensure system stability, iterative energy minimization was performed using optimization algorithms such as steepest descent and conjugate gradient until the maximum force was reduced below 100 kJ/mol/nm. The system was then equilibrated through positional-restrained dynamics under NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) ensembles. Equilibration involved 5000 steps under NVT conditions, followed by 50,000 steps under NPT conditions, both at 300 K. Once equilibrium was reached, a 50-nanosecond production MD run was conducted at 300 K and 1 bar pressure to explore the long-term dynamics of the system. During the simulation, various structural and dynamic properties, such as Root Mean Square Deviation (RMSD), Solvent Accessible Surface Area (SASA), Radius of gyration (ROG) and hydrogen bond were analyzed using GROMACS. These analyses provided detailed insights into the behavior of the protein-ligand complex with time. The workflow of the phytochemical screening and their computational validation is highlighted in Figure 2.

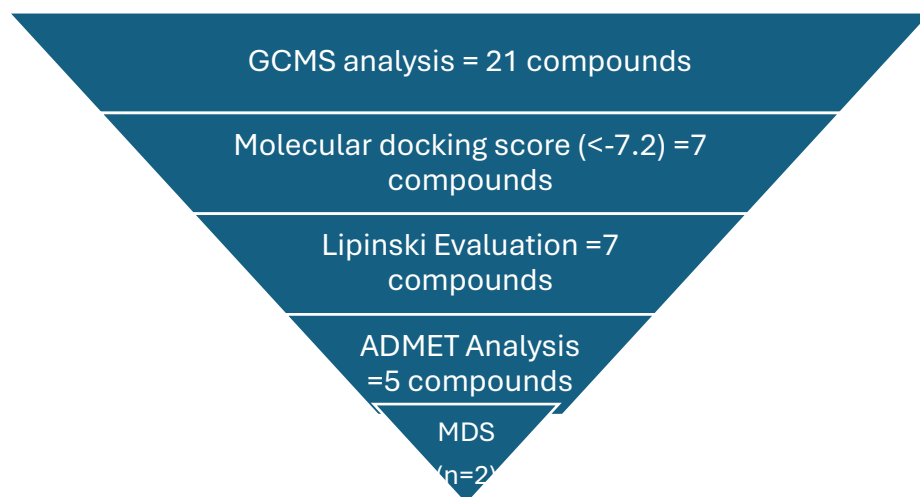


Figure 2: Workflow of phytochemical screening and validation procedure. MDS= molecular docking simulation

3. Results

3.1 Gas chromatography-mass spectrometer of ethanolic crude extract of *B. sapida* leaf

The GC-MS analysis of the ethanolic crude extract of *B. sapida* revealed the presence of twenty-one compounds (Table 1). The first compound to emerge from the GC was Benzoic acid, methyl ester with a retention time of 4.724 min, while the last to emerge was Bis (2-ethylhexyl) phthalate with a retention time of 19.452 min. The three predominant phytochemicals are D-Fructose, 3-O-methyl- (21.02%), Phytol (20.77%) and Hexadecanoic acid, ethyl ester (13.11%). These compounds were identified based on their peak area, retention time, molecular formula, molecular structure, molecular mass, and calculated fragments (Figure 3).

3.2 Molecular docking

The molecular docking via Autodock and MOE showed the binding affinity of the 7 lead bioactive compounds with the protein DHODH (Table 2). The structures of these compounds are listed in Figure 4. These lead compounds were selected based on their binding scores, Lipinski's rule of five parameters, and their ADMET properties²⁴. Phytol showed a consistently higher binding score of -11.9 Kcal/mol and -10.5 Kcal/mol in autodock and MOE respectively, The key interaction amino acids includes TYR528, ILE263, CYS276, ALA224, ALA225, LYS229, PHE278 followed by ethyl-9,12,15 octadecatrienoate with a score of -17.1 Kcal/mol and -10.3 Kcal/mol in Autodock and MOE, respectively with interactions involving CYS276, LYS229, ILE263, TYR528, ALA224, ALA225. Intriguingly, all were found to bind resolutely to the active residues of DHODH (Figure 5) with different bonds, including Van der Waal, Pi-Sigma, Alkyl, PI-Alkyl, conventional hydrogen Bond and carbon hydrogen Bond (Table 3).

Table 1: Compounds identified in the ethanolic leaf extract of *Blighia sapida* and their docking scores using GCMS

S/No	Name of components	Retention time	Area%	Molecular weight	Molecular formular	Binding energy (Autodock)	Binding energy (MOE)
1	Benzoic acid, methyl ester	4.724	0.75	136	C ₈ H ₈ O ₂	-5.4	-7.5
2	Benzoic acid, methyl ester	4.781	1.42	136	C ₈ H ₈ O ₂	-5.4	-7.5
3	D-Fructose, 3-O-methyl-	10.955	21.02	194	C ₇ H ₁₄ O ₆	-15.5	-7.5
4	aR-Turmerone	11.161	2.99	216	C ₁₅ H ₂₀ O	-7.8	-7.6
5	2-Methyl-6-(4-methylenecyclohex-2-en-1-yl)hept-2-en-4-one	11.533	1.41	218	C ₁₅ H ₂₂ O	-8.1	-7.8
6	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	12.220	1.06	196	C ₁₁ H ₁₆ O ₃	-7.6	-8.5
7	Neophytadiene	12.677	2.73	279	C ₂₀ H ₃₈	-7.0	-9.2
8	3-Eicosyne	13.049	1.42	279	C ₂₀ H ₃₈	-6.5	-9.6
9	Hexadecanoic acid, methyl ester	13.438	4.45	270	C ₁₇ H ₃₄ O	-15.6	-9.4
10	n-Hexadecanoic acid	13.765	7.46	256	C ₁₆ H ₃₂ O	-5.0	-9.3
11	Hexadecanoic acid, ethyl ester	13.999	13.11	284	C ₁₈ H ₃₆ O ₂	-7.4	-7.9
12	9,12-Octadecadienoic acid, methyl ester	14.800	1.79	294	C ₁₉ H ₃₄ O ₂	-5.8	-9.4
13	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	14.846	2.04	292	C ₁₉ H ₃₂ O ₂	-6.1	-9.8
14	Phytol	14.932	20.77	297	C ₂₀ H ₄₀ O	-17.4	-10.5
15	Methyl stearate	15.052	0.94	299	C ₁₉ H ₃₈ O ₂	-7.1	-7.1
16	9,12-Octadecadienoic acid (Z,Z)-	15.115	0.89	280	C ₁₈ H ₃₂ O ₂	-5.6	-9.4
17	Tridecanedial	15.167	2.61	212	C ₁₃ H ₂₄ O ₂	-4.9	
18	Linoleic acid ethyl ester	15.310	4.05	308	C ₂₀ H ₃₆ O ₂	-10.0	-9.8
19	Ethyl 9,12,15-octadecatrienoate	15.361	5.09	306	C ₂₀ H ₃₄ O ₂	-17.1	-10.3
20	Octadecanoic acid, ethyl ester	15.561	2.45	313	C ₂₀ H ₄₀ O ₂	-7.9	-7.9
21	Bis(2-ethylhexyl) phthalate	19.452	1.55	391	C ₂₄ H ₃₈ O ₄	-9.8	-9.5

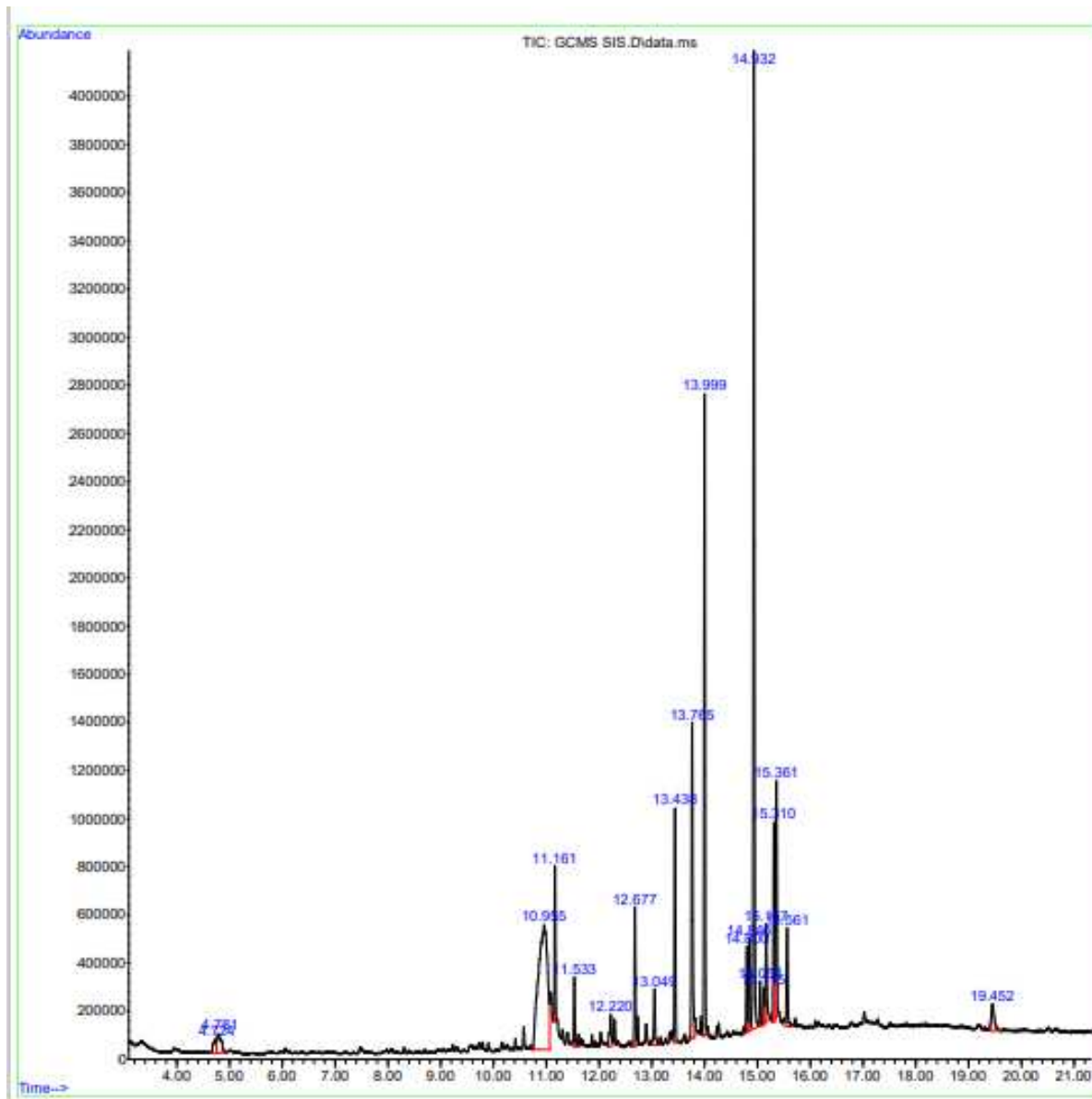


Figure 3: The chromatogram of GCMS analysis of ethanol extract of the ethanolic leaf extract of *Blighia sapida*

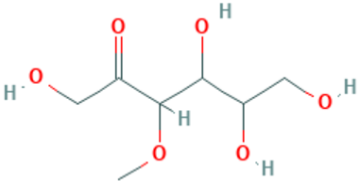
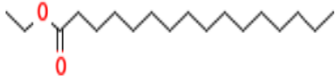
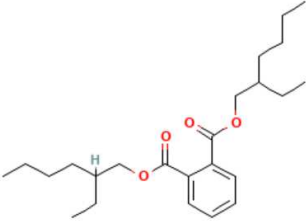
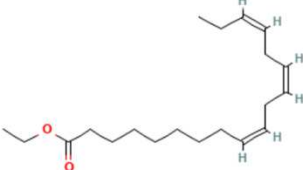
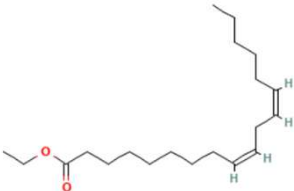
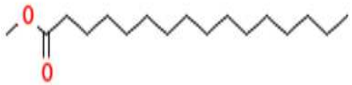
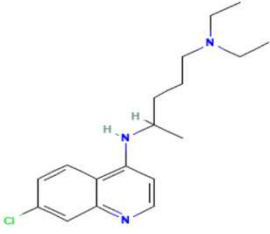
 D-Fructose, 3-O-methyl -	 Phytol	 Hexadecanoic acid, ethyl ester
 Bis(2-ethylhexyl) phthalate	 Ethyl 9,12,15-octadecatrienoate	 Linoleic acid ethyl ester
 Hexadecanoic acid, methyl ester	 Chloroquine	

Figure 4: Structures of hit bioactive compounds identified from *B.sapida* ethanolic crude extract and used for molecular docking analysis. (Photo courtesy: www.pubchem.ncbi.nlm.nih.gov).

262 Table 2: Molecular docking score for bioactive lead compounds of *B. sapida* with DHODH protein
 263 target.

S/No	Ligand	Docking score (Kcal/mol)	
		Autodock	MOE
1	D-Fructose,3-o-methyl-	-15.5	-7.5
2	Phytol	-17.4	-10.5
3	Hexadecanoic acid, ethyl ester	-7.4	-7.9
4	Bis(2-ethylhexyl) phthalate	-9.8	-9.5
5	Ethyl 9,12,15-octadecatrienoate	-17.1	-10.3
6	Linoleic acid ethyl ester	-10.0	-9.8
7	Hexadecanoic acid, methyl ester	-15.6	-9.4
8	Chloroquine	-7.2	-8.4

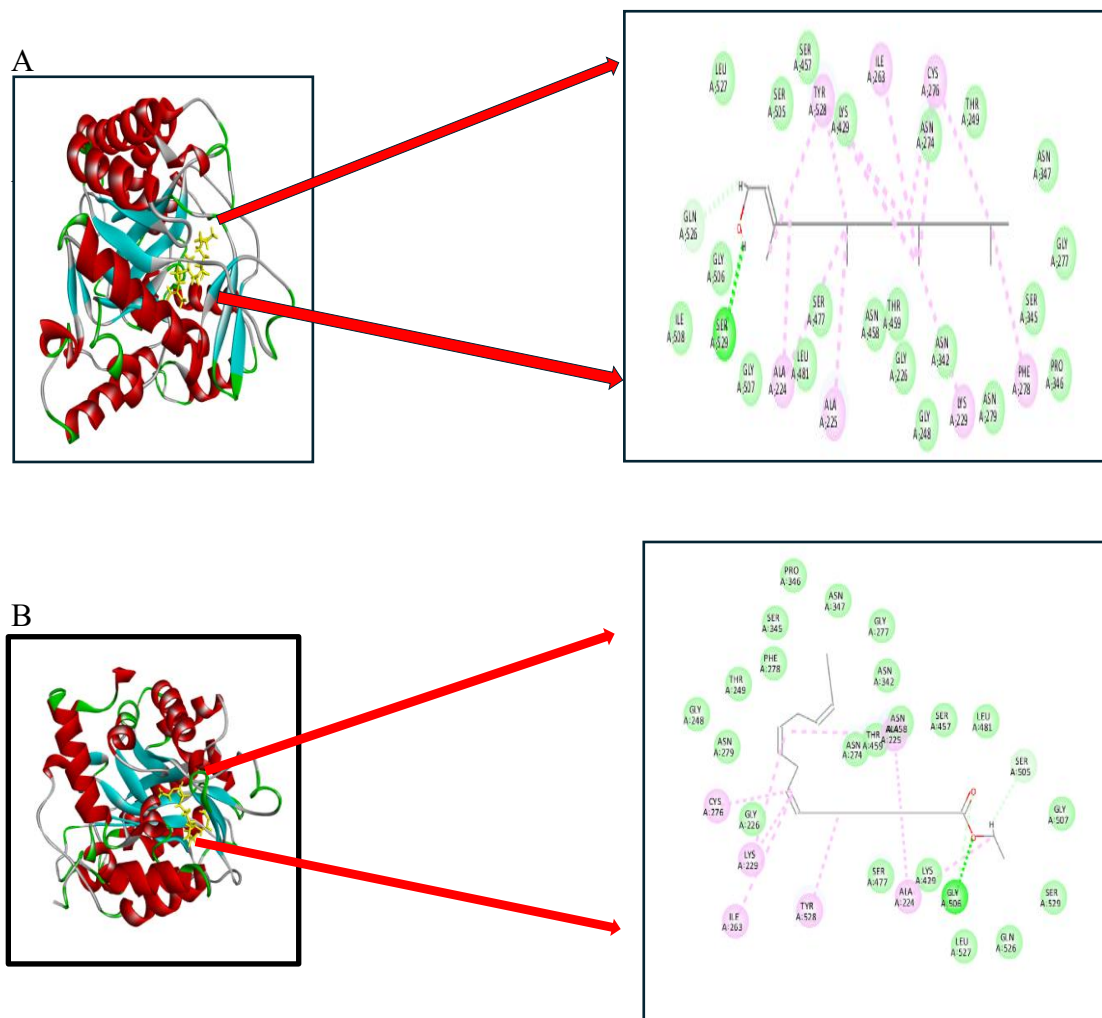
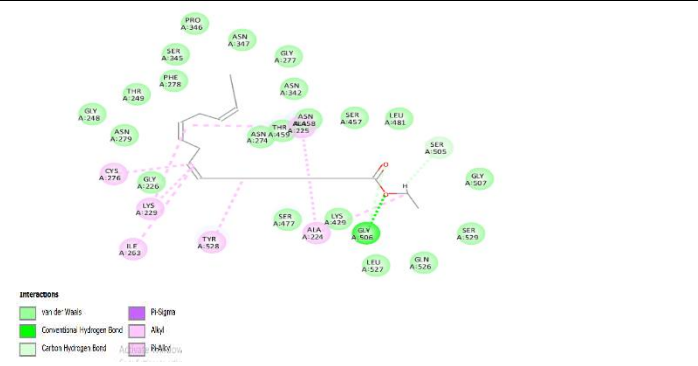
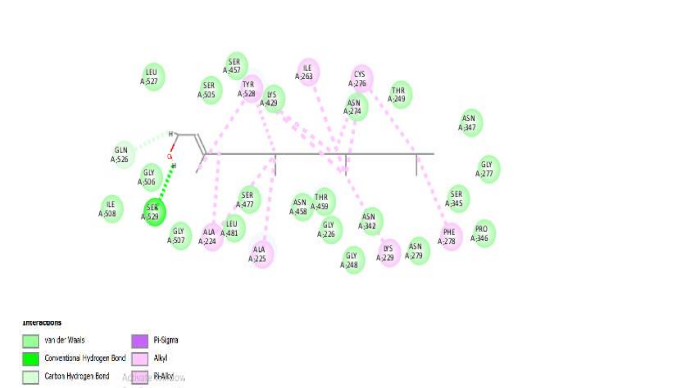
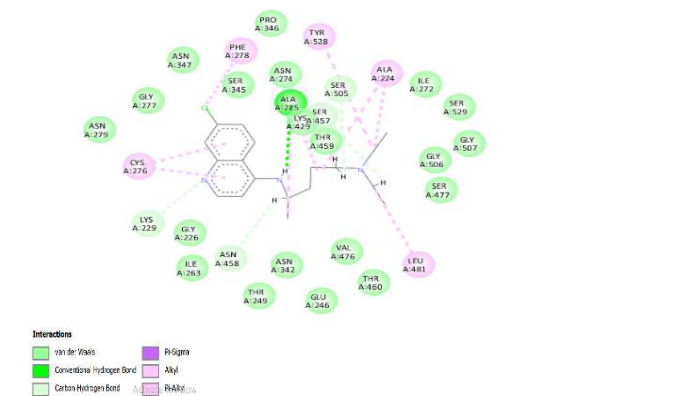


Figure 5: Structure of the lead compound in DHODH protein pockets and the 2D interaction of phytol and Ethyl 9,12,15-octadecatrienoate. (A) Phytol complex, (B) Ethyl 9,12,15-octadecatrienoate complex,

293

294 Table 3: 2D Interaction and list of interacting amino acids of lead compounds and the standard
295 drug

Compounds	Binding score	2D Interaction	H-bond Interaction	Hydrophobic Interaction
Ethyl 9,12,15 octadecatrienoate	-17.1		GLY506	CYS276, LYS229, ILE263, TYR528, ALA224, ALA225
Phytol	-11.9		SER529	TYR528, ILE263, CYS276, ALA224, ALA225, LYS229, PHE278
Chloroquine	-7.2		ALA225	PHE278, TYR528, ALA224, CYS276, LEU481

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299 3.3 Drug-Likeness

300 The drug-likeness of the lead compounds was assessed based on the physicochemical properties
301 listed in Table 4. The drug-likeness of seven compounds with the reference drug were assessed
302 using Lipinski's rule of five and associated descriptors, including molecular weight (MW),
303 calculated partition coefficient (MLOGP), number of hydrogen bond donors (HBD), hydrogen
304 bond acceptors (HBA), topological polar surface area (TPSA), bioavailability score, and skin
305 permeability (log Kp). The complete data are presented in Table 4.

306 All ligands analyzed in this study complied with Lipinski's rule, indicating favorable oral drug-
307 likeness characteristics. The molecular weight of the compounds ranged from 194.2 Da (D-
308 Fructose, 3-O-methyl-) to 390.6 Da (Bis(2-ethylhexyl) phthalate), remaining within the acceptable
309 threshold of 500 Da. Similarly, all compounds possessed fewer than five HBDs and fewer than ten
310 HBAs, fulfilling the corresponding Lipinski criteria. According to Lipinski's rule of five ²⁹, which
311 outlines the characteristics of potential drug candidates, all the lead compounds demonstrated
312 features indicative of their potential as therapeutic agents.

313 3.4 ADMET analysis

314 The predicted ADMET (Absorption, Distribution, Metabolism, and Excretion) pharmacokinetic
315 properties of these compounds, summarized in Table 5, offer valuable insights into their potential
316 therapeutic applications. All compounds displayed superior gastrointestinal (GI) absorption with
317 the exception of D-Fructose, 3-O-methyl- which shows a lower absorption compared to the control
318 (Table 5). All compounds also showed high Caco-2 membrane permeability (log Papp > 0.9 cm/s),
319 suggesting favorable absorption potential in the human body. Blood-brain barrier (BBB)
320 permeability suggests that most compounds can cross the BBB (log BB > 0.7), except D-Fructose,
321 3-O-methyl- (-1.013) and n-Hexadecanoic acid (-0.111), which are unlikely to reach the central
322 nervous system (CNS). Metabolism predictions assessed the interaction of the isolated compounds
323 with cytochrome P450 isoforms, focusing on their roles as substrates or inhibitors. None of the
324 compounds inhibited cytochrome P450 isoenzymes except CYP1A2 inhibitor; however, all the
325 lead compounds were identified as substrates for CYP3A4 isoenzymes, suggesting they may

influence metabolic pathways regulated by CYP3A4 with the exception of D-Fructose, 3-O-methyl- and n-Hexadecanoic acid.

Regarding excretion kinetics, Ethyl 9,12,15-octadecatrienoate had the highest total clearance score (2.134 log mL/min/kg), indicating efficient elimination, while D-Fructose, 3-O-methyl- exhibited the lowest clearance score (0.967log mL/min/kg), suggesting slower elimination compared to Ethyl 9,12,15-octadecatrienoate. Toxicity profiles suggest that chloroquine and n-Hexadecanoic acid are Ames-positive, indicating potential mutagenicity. Chloroquine is the only compound with hepatotoxicity concerns. Skin sensitization is observed for most compounds, except D-Fructose, 3-O-methyl- and Chloroquine.

Table 4: Drug-likeness prediction of the lead compounds from the extract of *B. sapida*

Ligands	Lipinski properties							
	MLOGP	MW	H-bond Donor (<5)	H-bond Acceptors (<10)	TPSA(A)	Lipinski rule	Bioavailability Score	log Kp (cm/s)
Phytol	5.25	296.5	1	1	20.23	Yes	0.55	-2.29
D-Fructose, 3-O-methyl-	-2.56	194.2	4	6	107.22	Yes	0.55	-9.41
Hexadecanoic acid, ethyl ester	4.67	284.5	0	2	26.3	Yes	0.55	-2.44
Bis(2-ethylhexyl) phthalate	5.24	390.6	1	2	52.6	Yes	0.55	-3.39
Linoleic acid ethyl ester	2.5	308.5	0	2	26.3	Yes	0.55	-5.04
Ethyl 9,12,15-octadecatrienoate	4.19	306.5	0	2	26.3	Yes	0.55	-2.77
Hexadecanoic acid, methyl ester	4.44	270.5	0	2	26.3	Yes	0.55	-2.71
Chloroquine	3.2	319.9	1	2	28.16	Yes	0.55	-4.96

Note: mlogP: molecular lipophilicity potential, MW: molecular weight, logkp: skin permeation, TPSA: Topological surface area, nrotb:number of rotatable bonds, RO5: Rule of five

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362 Table 5: ADMET pharmacokinetic properties of the lead compounds from extract of *B. sapida*

Parameters	Ethyl 9,12,15-octadecatrienoate	Phytol	Hexadecanoic acid, methyl ester	Chloroquine
GI absorption	92.747	90.643	92.335	89.95
Caco2 permeability	1.615	1.399	1.6	1.62
P-gp substrate	No	No	No	Yes
P-gp I inhibitor	No	No	No	No
P-gp II inhibitor	Yes	No	No	No
BBB permeability	Yes	Yes	Yes	Yes
CYP2D6 substrate	No	No	No	Yes
CYP3A4 substrate	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	Yes	Yes	Yes	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	No	No	No	No
CYP2D6 inhibitor	No	No	No	Yes
CYP3A4 inhibitor	No	No	No	No
Total Clearance (log ml/min/kg)	2.134	1.686	1.861	1.09
AMES toxicity	No	No	No	Yes
hERG I inhibitor	No	No	No	No
hERG II inhibitor	No	Yes	No	Yes
Hepatotoxicity	No	No	No	Yes
Skin Sensitisation	Yes	Yes	Yes	No

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371 Table 5: ADMET pharmacokinetic properties of the lead compounds from extract of *B. sapida*
 372 (Cont'd)

	Linoleic acid ethyl ester	n- Hexadecanoic acid	Hexadecanoic acid, ethyl ester	D- Fructose, 3-O- methyl-	Chloroquine
GI absorption	92.241	84.757	91.916	52.318	89.95
CaCo2 permeability	1.608	1.525	1.596	-0.24	1.62
P-gp substrate	No	No	No	No	Yes
P-gp I inhibitor	No	No	No	No	No
P-gp II inhibitor	Yes	No	No	No	No
BBB permeability	Yes	No	Yes	No	Yes
CYP2D6 substrate	No	No	No	No	Yes
CYP3A4 substrate	Yes	No	Yes	No	Yes
CYP1A2 inhibitor	Yes	No	Yes	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	Yes
CYP3A4 inhibitor	No	No	No	No	No
Total Clearance (log ml/min/kg)	2.08	1.918	1.912	0.967	1.09
AMES toxicity	No	Yes	No	No	Yes
hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	No	No	No	No	Yes
Hepatotoxicity	No	No	No	No	Yes
Skin Sensitisation	Yes	No	Yes	No	No

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3.5 Molecular dynamics simulation of the complexes

Molecular dynamics (MD) simulations were carried out for the protein target- Dihydroorotate dehydrogenase (DHODH) together with the standard drug chloroquine, and two promising DHODH inhibitors—Phytol and Ethyl 9,12,15-octadecatrienoate identified through docking screening. The simulations, spanning 50,000 picoseconds (ps), assessed parameters such as root-mean-square deviation (RMSD), Radius of gyration (ROG), Solvent accessible surface (SASA), and Hydrogen bond (H-bond).

Root Mean Square Deviation (RMSD) is a key parameter used to evaluate conformational changes in ligand-protein complexes over time. RMSD values provide crucial insights into the stability and structural consistency of the complexes formed between the compounds and the DHODH protein target throughout the simulation. As shown in Table 6, chloroquine exhibited the lowest average RMSD value (0.176 nm), followed by Phytol, then Ethyl 9,12,15-octadecatrienoate. This indicates that the Phytol-protein complex maintained a highly stable and well-aligned structure during the simulation, with minimal deviation from its initial conformation when compared to Ethyl 9,12,15-octadecatrienoate. Such stability reflects a strong and consistent interaction between Phytol and the DHODH protein (Figure 6A).

The stability of a protein in a biological system can also be evaluated using the radius of gyration (ROG), which gauges how compact a protein and ligand complex is. The graph spectra of the bound forms of DHODH in Figure 6B show a similar ROG pattern apart from the standard. Ethyl 9,12,15-octadecatrienoate complex and Phytol complex both had average gyrational radii of 1.92 nm (Table 6). The average ROG values of Ethyl 9,12,15-octadecatrienoate complex and Phytol complexes (1.92 nm) and the reference inhibitor (1.91 nm) are extremely similar. Therefore, it can be concluded that the structural compactness of the DHODH protein target is not distorted by the binding of the possible Ethyl 9,12,15-octadecatrienoate and phytol treatment candidates.

Compounds with lower Solvent Accessible Surface Area (SASA) values when bound to target proteins often indicate tighter binding and enhanced structural integrity, highlighting their potential as drug candidates³⁶. From table 6, Phytol stands out as a promising candidate, almost similar to the reference drug chloroquine, based on its favorable SASA value. Phytol's lower SASA value suggests a trend toward greater compactness and stability in its complex with the DHODH protein

than Ethyl 9,12,15-octadecatrienoate (Figure 6c). The comparison with Chloroquine underscores Phytol's potential advantages in structural dynamics and stability. While chloroquine remains a well-established standard, Phytol's comparable characteristics in protein-ligand interactions highlight its promise as a drug candidate.

Protein-ligand interaction depends on conventional hydrogen bonds (H-bonds) and their relative strength in a water environment³⁷. When an electron from a hydrogen-bond acceptor approaches an electron from a hydrogen-bond donor that is directly bonded to another electron, a hydrogen bond is formed³⁸. The examination of the intermolecular H-bond preserved between Ethyl 9,12,15-octadecatrienoate, Phytol, and chloroquine complexes is shown in Table 6 and Figure 6D. These findings demonstrate that, in comparison to the standard (1.05 nm), Phytol complex has the largest average intermolecular H-bond (2.02 nm) while Ethyl 9,12,15-octadecatrienoate displayed minimal hydrogen bond value (0.57)

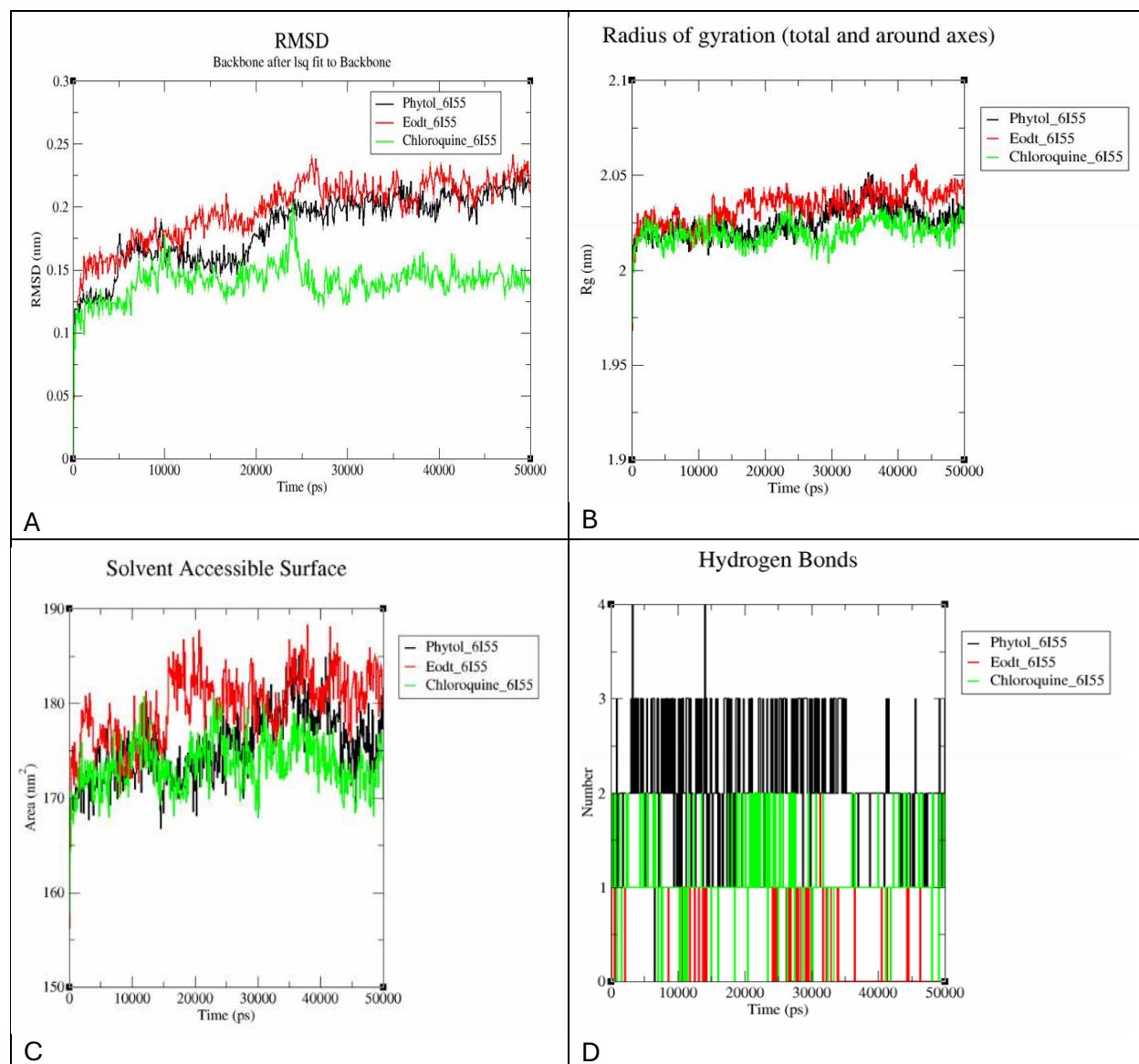


Figure 6: The superimposed graph spectrum of the lead compounds Phytol and Ethyl 9,12,15 octadecatrienoate (Eoxlt) and the reference compound (Chloroquine) in complex with DHODH. A-RMSF, B-ROG, C-SASA, D- Hydrogen bond

Table 6: Molecular dynamics average values of the RMSD, Rog, SASA and Hydrogen bond of the lead compounds and the standard complexes.

Complexes	Average RMSD values (nm)	Average Rog values (nm ²)	Average SASA values (nm ²)	Average Hydrogen bond values (nm)
Phytol complex	0.176	1.92	166.9	2.02
Ethyl 9,12,15-octadecatrienoate complex	0.191	1.92	171.1	0.57
Standard complex	0.135	1.91	166.2	1.05

4. Discussion

The area percentages of the 21 components identified in *Blighia sapida* ethanol extract vary widely, reflecting the variability of the relative abundance of each compound in the sample. D-Fructose, 3-O-methyl- (21.02%) and Phytol (20.77%) showed the highest area percentages, and Benzoic acid, methyl ester was the lowest (0.75%). The components identified span a variety of chemical families, including fatty acids, esters, alcohols, and terpenoids, which suggests a diverse chemical profile. Edewor, and Kazeem ³⁹, who worked on the methanol leaf extract of *B. sapida* identified many similar compounds such as phytol, hexadecenoic acid methyl ester and n-hexadecanoic acid, with Phytol as one of the predominant compounds. Phytol is a diterpene alcohol with recognized antioxidant, antimicrobial, and anti-inflammatory properties ⁴⁰. Similarly, D-fructose derivatives are known for their role in energy metabolism and potential prebiotic effects ⁴¹, suggesting therapeutic relevance of these two compounds.

Strong binding affinities to this target suggest potential inhibition of parasite replication ⁹. Phytol a diterpene alcohol and Ethyl 9,12,15-octadecatrienoate a fatty acid ester, demonstrated notable binding affinities in both Autodock (-11.9 Kcal/mol and 17.1 Kcal/mol) and MOE (-10.5 Kcal/mol and -10.3 Kcal/mol) respectively with hydrogen and hydrophobic bond interactions. Hydrogen bonds increase binding specificity and contribute to the orientation and stabilization of the ligand in the binding pocket, while hydrophobic interactions help in anchoring the non-polar regions of

the ligand within the enzyme. GLY506 found in flexible regions may allow ligand-induced conformational adjustments⁴², stabilize the ligand in the binding pocket, and enhance binding specificity. Hydrophobic residues like ILE263, ALA224, CYS276 are known to be crucial for the structural integrity of the binding site in DHODH⁴³, reinforcing the idea that this ligand can effectively bind and potentially inhibit the enzyme. Residues such as CYS276, LYS229, ALA224, ALA225 and TYR528 are shared with Phytol, indicating an overlap in binding regions and hotspots critical for inhibition. Targeting these conserved residues may enhance the efficacy and reduce the likelihood of resistance development⁴⁴. The interactions suggest that Ethyl 9,12,15-octadecatrienoate could act similarly to Phytol, potentially offering complementary inhibition or additive effects in combination therapies. Comparatively, lower affinities (-7.2 Kcal/mol and -8.4 Kcal/mol) showed by standard drug chloroquine in both Autodock and MOE respectively, suggesting that these natural compounds might outperform it in targeting DHODH. Thus, underscoring the potential of these ligands as complementary or antimalarial drug development.

The drug-likeness of the selected phytochemicals and the reference drug, chloroquine, was assessed using Lipinski's Rule of Five to determine their suitability as orally active drugs. According to Lipinski's criteria, an ideal oral drug candidate should possess a molecular weight (MW) ≤ 500 Da, MLOGP ≤ 5 , no more than 5 hydrogen bond donors (HBD), and no more than 10 hydrogen bond acceptors (HBA)²⁹. All the studied ligands in this work adhered to Lipinski's rule, suggesting favorable drug-like characteristics and are therefore promising candidates for drug development. Among the evaluated ligands, Phytol displayed a high MLOGP (5.25), slightly exceeding the Lipinski threshold. However, recent studies have demonstrated that such deviations are not uncommon among bioactive natural compounds and do not necessarily preclude oral activity, especially when compensated by other favorable ADME properties⁴⁵. The Lipinski rule specifies that, typically, an orally active drug should not have more than one violation of the criteria⁴⁶. With a low polar surface area (TPSA = 20.23 Å²) and moderate molecular weight (296.5 Da), Phytol is expected to exhibit good membrane permeability, as supported by its relatively high log Kp value (-2.29 cm/s), indicative of favorable skin permeability. Ethyl 9,12,15-octadecatrienoate, exhibit MLOGP values 4.19, molecular weights under 306.5 Da, and low TPSA values (26.3 Å²), aligning well with Lipinski's criteria and demonstrating high membrane permeability (log Kp values -2.77 cm/s). This compound may serve as effective lipophilic carriers or adjuvants in drug formulations, particularly for transdermal delivery systems⁴⁷. The molecular weights of all ligands

fell within or slightly above the Lipinski threshold of 500 Dalton²⁹, which predicts good bioavailability. TPSA predicts the ability of a molecule to cross biological barriers, with an optimal range being less than 140 Å² for oral drugs. All ligands exhibited TPSA values within the acceptable range (< 140 Å²), with D-Fructose, 3-O-methyl- having the highest TPSA (107.22 Å²), supporting its strong solubility. Bioavailability scores closer to 1 indicate better oral absorption potential⁴⁸. All ligands had scores of 0.55, suggesting moderate bioavailability potential. Skin permeability is essential for predicting dermal absorption in transdermal formulations: Phytol and Ethyl 9,12,15-octadecatrienoate derivatives had higher log Kp values, suggesting better potential for transdermal delivery.

The ADMET profiling of phytochemicals is a critical step in early drug discovery to ensure pharmacokinetic efficiency and safety. Ethyl 9,12,15-octadecatrienoate and Phytol exhibited high gastrointestinal (GI) absorption (92.747% and 90.643%, respectively), surpassing that of chloroquine (89.95%). These findings indicate a promising oral bioavailability profile for these compounds. Unlike chloroquine, a known P-gp substrate, all *B. sapida*-derived compounds were non-substrates, suggesting lower susceptibility to P-gp-mediated efflux and potentially improved intracellular retention⁴⁹. Only ethyl 9,12,15-octadecatrienoate and linoleic acid ethyl ester exhibited inhibition of P-gp II, implying possible interference with P-gp mediated transport pathways. Ethyl 9,12,15-octadecatrienoate and phytol, demonstrated positive blood brain barrier (BBB) permeability, a characteristic shared with chloroquine. Thus, preventing the central nervous system (CNS) from toxins and pathogens in the blood. Total clearance values provide insights into the excretion profile of compounds. The highest clearance rates were noted for ethyl 9,12,15-octadecatrienoate (2.134 log ml/min/kg) and phytol (1.686), suggesting rapid systemic elimination compared to chloroquine (1.09), which may influence dosing frequency and systemic exposure⁵⁰. Toxicity predictions revealed favorable profiles for the majority of the phytochemicals. Unlike chloroquine, which showed AMES toxicity, hepatotoxicity, and hERG II inhibition, Ethyl 9,12,15-octadecatrienoate and phytol from *B. sapida* were non-mutagenic and non-hepatotoxic.

The molecular dynamics simulation data provided valuable insights into the structural stability and interaction patterns of the Phytol, Ethyl 9,12,15-octadecatrienoate (EODT), and Chloroquine (standard) complexes with the target protein. The standard complex exhibited the lowest average RMSD (0.135 nm), indicating greater conformational stability and minimal deviation from the

initial conformation, while Phytol and EODT complexes exhibited slightly higher RMSD values of 0.176 nm and 0.191 nm, respectively. Despite the slight deviation, both values remained within an acceptable stability range for biologically relevant complexes⁵¹. Notwithstanding, all complexes maintained similar compactness, as evidenced by consistent Rg values, indicating that ligand binding did not induce substantial unfolding or compaction of the protein structure. Notably, the EODT complex maintained a consistent Rog profile, similar to Phytol and Chloroquine, highlighting that the intrinsic flexibility of the protein was preserved post-binding. These findings are aligned with recent literature emphasizing the role of stable Rg values in protein-ligand complex integrity⁵². The EODT complex also displayed the highest solvent accessible surface area, suggesting more solvent exposure and potential instability in the protein-ligand interface. These findings are consistent with studies done by Singh *et al.*⁵³ correlating SASA with ligand-binding efficiency.

Hydrogen bond analysis further distinguished the binding stability and specificity⁵⁴ of the ligands. The Phytol complex formed the highest average number of hydrogen bonds (2.02), surpassing both EODT (0.57) and the standard (1.05). This reflects Phytol's superior binding strength and interaction consistency with the protein, which is a key indicator of potential biological efficacy⁵⁵. Although the standard ligand demonstrated optimal structural stability, the strong and consistent hydrogen bonding observed in the Phytol complex suggests it may serve as a promising bioactive candidate. Conversely, the limited hydrogen bonding and higher flexibility of the EODT complex may necessitate further optimization to enhance its binding characteristics.

These results indicate that Phytol and Ethyl 9,12,15-octadecatrienoate have significant potential for pharmacological applications, given their strong interactions with the protein target. Notably, this aligns with the molecular docking analysis, where these ligands demonstrated superior binding affinity and drug-likeness compared to other docked ligands. However, further work needs to be done using other *Plasmodium falciparum* target proteins.

5. Conclusion

The GCMS analysis in this study showed to have newly discovered eighteen new compounds when compared to previous study. The molecular docking analysis showed that Phytol, followed by Ethyl 9,12,15-octadecatrienoate, present in the plant, helps to reduce the activities of DHODH necessary for parasite metabolism and survival. Its ability to disrupt critical metabolic pathways positions it as a valuable natural resource in the fight against malaria. The pharmacokinetic and toxicity profiles emphasize their therapeutic potential. The compounds demonstrated non-hepatotoxicity, efficient clearance, good GI absorption, and favourable blood-brain barrier penetration, making them suitable candidates for malaria treatment.

With the MD simulation parameters, Phytol emerges as a promising candidate due to its strong hydrogen bonding interactions and consistent structural metrics. With further development, it may provide a cost-effective and sustainable alternative to synthetic antimalarials, particularly in regions heavily burdened by malaria. These findings pave the way for further in vitro and in vivo studies to validate the therapeutic potential of these phytochemicals.

Data availability

All the data has been included in the manuscript

Funding

No funding was received for this work

Conflicting Interest

The authors declare no conflicting interest.

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