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Antiplasmodial Activities and Molecular Docking Study of the GC-MS Identified Bioactive Compounds in *Daniella oliveri* (Rolf) Hutch. and Dalziel, and *Morinda lucida* Benth Leaves.

Muazu Muhammed[1]*, Deborah Madi Dibal[2], Yahaya Abdullahi Umar[2], Anthony Philip Vantsawa[2], Yahaya Yakubu[3].

[1]Department of Environmental Science, National Open University of Nigeria (NOUN), Jabi, Abuja, Nigeria.
[2]Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria.
[3]Department of Pure and Applied Chemistry, Kaduna State University, Kaduna, Nigeria.

Abstract

Malaria is a leading cause of death in many developing countries where young children and pregnant women are mostly affected. The rapid emergence and spread of resistant *Plasmodium falciparum* to artemisinin derivatives and other conventional antimalarial drugs necessitated the study of bioactive compounds, antiplasmodial potentials, and synergistic effects of *Daniellia oliveri* and *Morinda lucida leaves*. This study aimed to determine the antiplasmodial activities and molecular docking of the GC-MS identified bioactive compounds in *Daniella oliveri* and *Morinda lucida* leaves. Aqueous crude extraction of pulverized *Daniellia oliveri* leaves (ADOE), *Morinda lucida* leaves (AMLE), and combined *Daniellia oliveri* and *Morinda lucida* leaves (ADME) was performed. Fractionation of the aqueous extract of the combined leaves (ADME) was performed to obtain the methanol (MFC), ethanol (EFC), and ethyl acetate (EAFC) fractions. GC-MS analysis was used to identify bioactive compounds in the three aqueous crude extracts, in addition to antiplasmodial and molecular docking studies. The GC-MS analysis identified a total of 65 bioactive compounds in the combined leaves extract, MFC was the most potent, with the highest antiplasmodial activity. Molecular docking studies identified erucic and linolelaidic acids as the two bioactive compounds with the highest binding affinity for the targeted *Pb*LDH receptor, indicating the highest antiplasmodial activities of the two compounds. The purified MFC contained compounds identified to possess the antiplasmodial potentials

Keywords: Antiplasmodial activity, molecular docking, GC-MS, Daniellia *oliveri*, *Morinda lucida.* Eruric acid

*Corresponding author:
muazmed@gmail.com;
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Introduction

Malaria is a leading cause of death in many developing countries, where young children and pregnant women are the most affected groups (CDC, 2021). Nigeria loses over $1.1 billion annually to malaria prevention (Chukwuma, 2022). The rapid emergence and spread of resistant *Plasmodium falciparum* to artemisinin derivatives and all conventional antimalarial drugs necessitated the study of bioactive compounds and the antiplasmodial potential of *Daniellia oliveri* and *Morinda lucida* leaves (Muazu *et al*., 2021).

Despite the use of a non-commercially available vaccine called RTS,S (Mosquirix) against malaria among children in sub-Saharan Africa and in other regions with moderate to high *P. falciparum* malaria transmission (WHO, 2022), improved diagnostic techniques, treatment measures, and increased preventative activities such as the use of insecticide-treated nets (ITNs, including long-lasting insecticidal nets and insecticidal-treated bed nets), indoor residual spraying (IRS), prophylactic drugs (PD), and untreated nets (UN) against malaria (Wangdi, 2018), the number of deaths from malaria increased from 187,437 in 2019 to 199,689 (6.5% increase) in 2020 (Ode, 2022), resulting in an estimated loss of US$12 billion a year in Africa (Greenwood *et al*., 2005).

Daniellia oliveri (*D. oliveri*) is a medium-sized, deciduous tree growing to a height of 25 m (80 ft) or more, sometimes seen with a twisted trunk that is up to 200 cm (80 in) in diameter, a broad, flat-topped crown, and usually lacks branches on the lowest 9 m (30 ft) of trunk (Fern, 2019). Different parts of *D. oliveri* contain liquid oleoresin, which has been traditionally used for the management of various diseases in Africa (Nwuche and Eze, 2009). Flavonoids, saponins, cardiac glycosides, phenols, tannins, and alkaloids have been identified in *D. oliveri* leaves. Saponins and flavonoids are recognized as anti-inflammatory and antioxidant compounds (Lawal *et al*. 2022).

Morinda lucida (*M. lucida*) Benth (Rubiaceae) is an ethnomedicinal plant that has been extensively used in traditional medical practice for the treatment of malaria, diabetes, hypertension, inflammation, typhoid fever, cancer, cognitive disorders, sickle cell disease, trypanosomiasis, onchocerciasis, and various fevers (Adewole, 2021). The antimalarial analysis conducted on albino mice showed promising effects of *M. Lucida* extract as an antimalarial agent (Adebayo *et al*., 2020).

Pharmacological activities such as cytotoxic, hepatoprotective, anthelmintic, antibacterial cardiovascular, anti-ulcer, anti-spasmodic, antioxidant/anti-radical, anti-nociceptive, antimicrobial, anti-inflammatory, anti-diarrheal and anti-diabetic activities of *D. oliveri* have been reported (Muhammad, 2017). Synergistically, leafy decoctions of *Daniella oliveri* and *Cassytha filiformis* are used for the treatment of fever (Igoli *et al*., 2005), and *M. lucida* and *Alstonia boonei* in combination possess antiplasmodial activities and are used in malaria treatment (Afolabi and Abejide, 2020). This study aimed to investigate the bioactive compounds and antiplasmodial potential of extracts from the combined *leaves of Daniellia oliveri and Morinda lucida*.

Materials and Methods

Preparation of Leaves

Daniellia oliveri and M. lucida leaves were collected in March 2024 from the Plant Science and Technology Garden, Prince Abubakar Audu University, Anyigba, Kogi State, North-Central Nigeria. The leaves were identified and verified by Prof. G.A. Ajibade is an expert in the Department of Biological Sciences, Nigerian Defence Academy, Kaduna State, North-Western Nigeria. Voucher specimens were deposited with voucher numbers NDA/BIOH/202424 for *D. oliveri* leaves and NDA/BIOH/202425 for *M. lucida* leaves. The leaves were air-dried for three weeks at room temperature and pulverized with a mortar and pestle to obtain powdered material.

Aqueous Crude Extraction

The decoction method of extraction was used to prepare the aqueous crude extract of *D. oliveri, M. lucida* leaves, and the leaves combination by dissolving 100 g of the pulverized *D. oliveri* leaves, *M. lucida leaves,* or combination of equal weights (50 g of each plant powder) in 4000 mL of distilled water and allowed to boil for 1 h (Chaniad *et al*., 2022). Each mixture was filtered using Whatman filter paper (Whatman, Buckinghamshire, England) while rotary evaporator (Stuart, SO1, UK) set at 40^{o}C was used for drying the extracts (Kalay *et al*., 2020), and the percentage (%) yield was calculated,

Fractionation of Aqueous Crude Extracts

The method described by Abubakar and Haque (2020) was used for the fractionation of the aqueous crude extracts. Methanol, ethanol, and ethyl acetate were successively used to partition the aqueous crude extracts of the combination into three fractions using a separating funnel.

Fractionation was achieved by dissolving the dried extract in 250 mL of distilled water and dispensing it into a separating funnel. The mixture was thoroughly shaken and allowed to settle, and successively eluted with ethyl acetate, ethanol, and methanol in that order to obtain their respective fractions (Heftmann *et al*., 1992, Rimando *et al*., 2001, Sasidharan *et al*., 2011, Ingle *et al*., 2017)).

The extracts and fractions were concentrated using water bath (BIOBASE WT-60, China) at 50 ^{0}C (Okoro *et al*.,

2014), and were used for the determination of antiplasmodial activity (Mohammed *et al.,* 2014; Debela *et al.,* 2016; Adebayo *et al*., 2020).

GC-MS Identification of Compounds in Combined Leaves

The method of Olivia *et al.* (2021) was adopted for gas chromatography-mass spectrometry (GC-MS) analysis. Using a combined 7890A gas chromatograph system (GCMS-QP2010 PLUS SHIMADZU, JAPAN) and mass spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 µm, film thickness 0.25 µm), interfaced with 5675C Inert MSD (Mass Selective Detector) with Triple-Axis detector, and the relative percent amount of each compound present in aqueous crude extract of the combined leaves were determined by comparing its average peak area to the sum of all areas. Data of the identified components were collected using MS solution software based on their retention indices, and the mass spectrum was interpreted in comparison with the information in the National Institute of Standards and Technology (NIST) database.

Antiplasmodial Studies

A total of 105 albino mice of both sexes (18–25 g) were randomly selected along with a donor mouse containing 20% parasitemia and procured from the Animal House of the Institute of Advanced Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were acclimatized for seven days prior to the antiplasmodial study. With the aid of chloroform, the donor mouse was subjected to anesthesia, and 0.2 ml of blood containing *P. berghei-infected* erythrocytes was humanely collected through cardiac puncture and used in 4.8 ml of normal saline. The blood sample was serially diluted to 1 × 107, and through the intravenous route, the mice in the treatment groups were infected with 0.2 ml of 1 × 107 standard inoculum of the chloroquine-sensitive *P. berghei*-infected erythrocytes (Alo *et al*., 2018).

Rane's (Curative) test (Adigo Shibeshi *et al*., 2021) was used to determine the curative potential of the aqueous crude extracts and fractions following the method described by Fidock *et al*. (2004) for established infections. Mice (5 mice per group) were infected intravenously with 0.2 ml of 1 × 107 standard inoculum of chloroquine-sensitive *P. berghei*-infected erythrocytes from the donor mouse on the first day (day 0). Mice in the normal control group (NCTRL) were not infected with *P. berghei*.

After (72) hours later (day-3), mice in the positive control group (Ghq) were administered chloroquine (5 mg/kg) for five consecutive days (days 3–7). Mice in the negative control (UCTRL) and normal control (NCTRL) groups were administered 0.2 ml of normal saline for five consecutive days (Days 3–7).

Mice treated with the aqueous crude extracts and fractions (3 groups each) received 200, 400 and 800 mg/kg of the extracts or fractions, once daily for five consecutive days (Day 3 to Day 7).

Determination of parasitemia

From the 3rd to 7th day post-infection, parasitemia levels were determined daily in all groups. Blood samples were collected from the tail of the mice and dropped onto a microscopic slide. Thin and thick blood smears were prepared, air-dried at room temperature, fixed with absolute methanol for 30 s, and stained with 10% Giemsa stain for 15 min. The slides were allowed to air-dry, and with the aid of immersion oil, using X100 objective lens on a light microscope (OLYMPUS, China), both the parasitized and total red blood cells were examined and counted. The percentage parasitemia was determined by counting a minimum of 100 RBC per field with a minimum of three fields per slide (Milka *et al*., 2023).

The percentage (%) parasitemia suppression was calculated by comparing the parasitemia in the negative control group with that in the treated mice (test groups) as follows:

% Parasitemia Suppression =

(A – B / A) x 100

Where; A = Mean percentage parasitemia in negative control group,

B = Mean percentage parasitemia in the test group (Getu and Solomon, 2020).

Molecular docking analysis

Following the antiplasmodial studies, the bioactive compounds identified in the combined extract (ADME) after GC-MS analysis were subjected to molecular docking according to the method described by Enenebeaku *et al*. (2021), using antimalarial protein targets. *P. berghei* lactate dehydrogenase (*Pb*LDH), a key enzyme that catalyzes the synthesis of lactate from pyruvate in *Plasmodium* species, with the molecular target proteins (ID: 1OC4) obtained from protein databank (PDB) (www.rcsb.org), and minimized using UCSF Chimera 1.14. The structural data files (SDF) of all compounds were obtained from the PubChem database.

The 63 bioactive compounds identified through GC-MS analysis of ADME were used as ligands. The binding affinities of the ligands for the protein targets were determined using AutoDock Vina from PyRx (Johnson *et al*., 2020). The docking results, which are the molecular interactions between proteins and ligands, were viewed using BIOVIA Discovery Studio 2020.

Statistical Analysis

The method adopted by Bantie *et al*. (2014) was utilized for data analysis using Microsoft Excel, SPSS, Graph Pad, and SAS 9.12 software. One-way analysis of variance (ANOVA) was used to detect treatment effects. Pearson correlation was used to evaluate the relationship between the measured parameters. Duncan's multiple range test (DMRT) was used to separate the means. All data were analyzed at a 95% confidence interval, and $P < 0.05$ was considered statistically significant.

Results

GC-MS analysis of the combined leaves extract

Following the GC-MS analysis, a total of 63 bioactive compounds were identified in the combination extract (ADME) in relation to the molecular weight and retention index shown in (Table .1), and the chromatogram of the GC-MS analysis (Figure 1).

Antiplasmodial Activities of the Aqueous Crude Extracts and Fractions

The percentage suppression indicated that treatment with chloroquine (5 mg/kg) reduced parasitemia by 100%, while ADME and MCF (800 mg/kg) had 97.87% and 99.1% suppression, respectively. From day 4, the percent suppression of parasitemia by the extracts and fractions was time- and dose-dependent (Tables 2–7).

Molecular Docking Studies

Molecular docking studies showed good binding affinity for all compounds with the targeted *Pb*LDH receptor, ranging between (-6.6 kcal/mol and -4.2 kcal/mol). Quinine showed a higher binding affinity of 6.6 kcal/mol than chloroquine -6.1 kcal/mol. Quinine and chloroquine exhibited higher binding affinities than the other compounds (complexes). Complex 36 (erucic acid) and Complex 29 (linolelaidic acid) possessed equal binding affinity, indicating the highest binding affinity (-5.9 kcal/mol) of all the GC-MS identified bioactive compounds studied using molecular docking. However, Complex 5 (cyclopropane) produced the lowest binding affinity (-2.5 kcal/mol), indicating the highest binding energies (Table 8). Complex 36 (erucic acid) and Complex 29 (linolelaidic acid), in addition to quinine and chloroquine, were further visualized to obtain more views of the residual interactions regarding their binding poses.

The binding poses for quinine showed lower binding energy (- 6.6 kcal/mol) (Figure 2 a, b) than those of chloroquine (-6.1 kcal/mol) (Figure 3 a, b), which were visualized for an in-depth understanding of the different forms of residual interactions created with the active pocket of the *Pb*LDH receptor. In comparison, quinine and chloroquine exhibited lower binding energies than all the GC-MS-identified bioactive compounds. The binding poses of erucic acid (Figure 4 a, b, c) and linolelaidic acid (Figure 5 a, b, c) were shown to possess equal and lowest binding energies (-5.9 kcal/mol).

The quinine molecule formed only one (1) conventional hydrogen bond interaction with the amino acid residues of (TYR A: 175) along with numerous interactions that included four (4) alkyls (ILE A: 239); (VAL A: 248); (ALA A: 249) and (ARG A: 171) residues (Figure 2 a, b).

The chloroquine molecule formed only one (1) conventional hydrogen bond interaction with the amino acid residues of (THR B: 97) along with numerous interactions that includes two (2) Carbon Hydrogen Bond (ASP B: 53) and (MET B: 30); Pi Donor Hydrogen Bond (PHE B: 100) and Akyl (ILE B: 31) residues (Figure 3 a, b).

The eruric acid molecule with the *Pb*LDH receptor formed two (2) conventional hydrogen bonds with the amino acid residues of (ARG B: 171) and (ARG B:109) at different bond distances, with other interactions that also include two alkyls (ALA B:98); (ILE B:54) residues (Figure 4 a, b, c).

The linolelaidic acid molecule with the *Pb*LDH receptor formed three (3) conventional hydrogen bonds with the amino acid residues of (ARG A: 171); (HIS A:195); (ARG A: 109) at different bond distances, with other interactions that also includes two (2) Alkyl (ALA A:98); (ILE A:54) residues (Figure 5 a, b, c).

Table 1: Compounds identified by GC-MS in the combined leaves extract

S/N	Compounds	Molecular Weight	Retention Index
1	Decylene	140	1005
2	Neodene 10	140	1005
3	1-Nonanol	144	1159
4	Pelargonic alcohol	144	1159
5	Cyclopropane	140	979
6	Undecene, (E)-	154	1123
7	alpha. -Farnesene	204	1458
8	trans-. alpha. -Bergamotene	204	1430
9	Myristic acid	242	1680
10	Metholeneat 2495	242	1680
11	Metholeneat 2495	242	1680
12	Decanoic acid	186	1282
13	methyl ester	186	1282
14	Capric acid	186	1282
15	Myristic acid	228	1769
16	1-Tridecanecarboxylic acid	228	1769
17	Palmitic acid	256	1968
18	Pentadecanecarboxylic acid	256	1968
19	Stearic acid	284	2167
20	Hydrofol Acid 150	284	2167
21	Hystrene S-97	284	2167
22	Margarinic acid	270	2067
23	Margaric acid	270	2067
24	Methyl palmitoleate	268	1886
25	Cyclopropaneoctanoic acid	282	1941
26	Palmitic acid,	270	1878
27	Nonadecanoic acid	298	2266
28	Pentadecanoic acid	242	1869
29	Linolelaidic acid	294	2093
30	Eicosadienoic acid	322	2292
31	Elaidic acid	296	2085
32	Kemester 9718	298	2077
33	Metholene 2095	186	1282

34	Heneicosanoic acid	340	2375
35	Oleic Acid	282	2175
36	Erucic acid	338	2572
37	Aqua Cera	372	2694
38	Atlas G 2146	372	2694
39	Cerasynt	372	2694
40	Cerasynt Special	372	2694
41	Clindrol SDG	372	2694
42	Pentadecanecarboxylic acid	256	1968
43	Hexadecanoic acid	256	1968
44	n-Hexadecoic acid	256	1968
45	1-Pentadecanecarboxylic acid	256	1968
46	Hexadecanohydrazide	270	2255
47	Oxalic acid	342	2344
48	dodecyl hexyl ester	342	2344
49	4-n-Propylheptadecane	282	1945
50	Erucic acid	338	2572
51	cis-13-Docosenoic acid	338	2572
52	Oleic Acid	282	2175
53	Z-9-Tetradecenal	210	1609
54	Z-9-Tetradecenol	210	1609
55	Brassidic acid	338	2572
56	Methyl erucate	352	2483
57	15-Tetracosenoic acid	380	2682
58	15-Tetracosenoic acid	380	2682
59	(Z)-Methyl nervonate	380	2682
60	Octyl 10-undecenoate	296	2067
61	Brassidic acid	338	2572
62	Hexadecenal	238	1808
63	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	268	1877

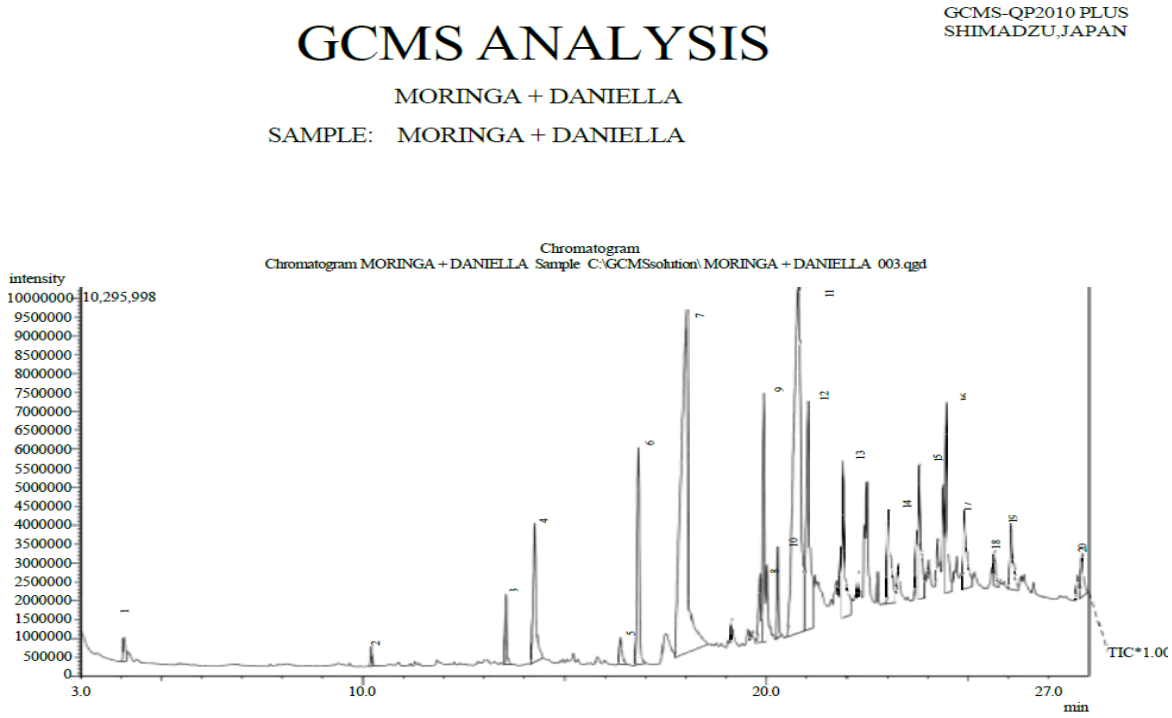

Figure 1: Chromatogram of GC-MS Identified Compounds

Table 2: Percentage parasitemia suppression by the Aqueous Crude Extract of *D. oliveri* leaves. (ADOE)

Days	Chq (5 mg/kg)	untreated (UCTRL)	Normal (NCTRL)	200 mg/kg ADOE	400 mg/kg ADOE	800 mg/kg ADOE
Day 3	-3.87	8.432	0	-9.35	2.75	-6.07
Day 4	80.88	10.41	0	19.23	41.02	52.62
Day 5	100	10.696	0	49.96	62.38	80.98
Day 6	100	11.226	0	62.78	80.71	91.79
Day 7	99.56	11.34	0	81.29	80.90	92.95

Chq: Chloroquine, UCTRL-: infected but untreated control; NCTRL: Normal uninfected Control, ADOE: Aqueous Crude Extract of *D. oliveri* leaves. (n=3).

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Table 3: Percentage parasitemia suppression by the Aqueous Crude Extract of *M. lucida* leaves. (AMLE**)**

Days	Chq (5 mg/kg)	untreated (UCTRL)	Normal (NCTRL)	200 mg/kg AMLE	400 mg/kg AMLE	800 mg/kg AMLE
Day 3	-3.87	8.432	0	-0.09	-3.98	-0.81
Day 4	80.88	10.41	0	24.88	42.44	48.57
Day 5	100	10.696	0	57.14	67.26	80.22
Day 6	100	11.226	0	69.70	80.47	91.40
Day 7	99.56	11.34	0	86.70	94.50	87.90

Chq, Chloroquine, UCTRL, infected but untreated control; NCTRL: Normal uninfected Control, **AMLE**: Aqueous Crude Extract of *M. lucida* leaves. (n=3).

Table 4: Percentage parasitemia suppression by the Aqueous Crude Extract of the combined D. oliveri and *M. lucida* leaves. (ADME)

Days	Chq (5 mg/kg)	untreated (UCTRL)	Normal (NCTRL)	200 mg/kg ADME	400 mg/kg ADME	800 mg/kg ADME
Day 3	-3.87	8.43	0	1.73	-3.68	-4.58
Day 4	80.88	10.41	0	23.36	45.96	47.65
Day 5	100	10.70	0	45.83	76.96	81.06
Day 6	100	11.23	0	73.74	83.68	91.41
Day 7	99.56	11.34	0	87.37	92.12	97.87

Chq: Chloroquine; UCTRL: infected but untreated control; NCTRL: Normal uninfected Control, **ADME**: Aqueous Crude Extract of the combined D. oliveri and *M. lucida* leaves. (n=3).

Table 5. Percentage parasitemia suppression by the methanol fraction of the combined leaves. **(MFC)**

Days	**Chq (5 mg/kg)**	**untreated (UCTRL)**	**Normal (NCTRL)**	**200 mg/kg MFC**	**400 mg/kg MFC**	**800 mg/kg MFC**
Day 3	-3.87	8.43	0	0.52	2.32	10.86
Day 4	80.88	10.41	0	25.09	27.44	99.84
Day 5	100	10.70	0	47.40	66.04	99.75
Day 6	100	11.22	0	76.38	80.60	99.87
Day 7	99.56	11.34	0	89.33	96.08	99.81

Chq: Chloroquine, UCTRL: infected but untreated control; NCTRL: Normal uninfected Control, **MFC**: Methanol fraction of the combined leaves. (n=3).

Table 6: Percentage parasitemia suppression by ethanol fraction of the combined leaves. (EFC)

Days	Chq (5 mg/kg)	untreated (UCTRL)	Normal (NCTRL)	200 mg/kg EFC	400 mg/kg EFC	800 mg/kg EFC
Day 3	-3.87	8.432	0	-2.61	1.52	9.82
Day 4	80.88	10.41	0	24.86	26.74	53.29
Day 5	100	10.69	0	46.39	63.39	83.69
Day 6	100	11.23	0	75.81	89.06	93.18
Day 7	99.56	11.34	0	88.15	94.44	96.10

Chq, Chloroquine, UCTRL-, infected but untreated control; NCTRL: Normal uninfected Control, **ECF**: Ethanol fraction of the combined leaves. (n=3).

Table 7: Percentage parasitemia suppression by the ethyl acetate fraction of the combined leaves. (EAFC)

Days	Chq (5 mg/kg)	untreated (UCTRL)	Normal (NCTRL)	200 mg/kg EAFC	400 mg/kg EAFC	800 mg/kg EAFC
Day 3	-3.87	8.432	0	-3.84	0.14	8.21
Day 4	80.88	10.41	0	24.67	25.51	52.76
Day 5	100	10.69	0	45.38	63.01	83.08
Day 6	100	11.22	0	74.42	88.03	91.91
Day 7	99.56	11.34	0	87.16	93.17	95.86

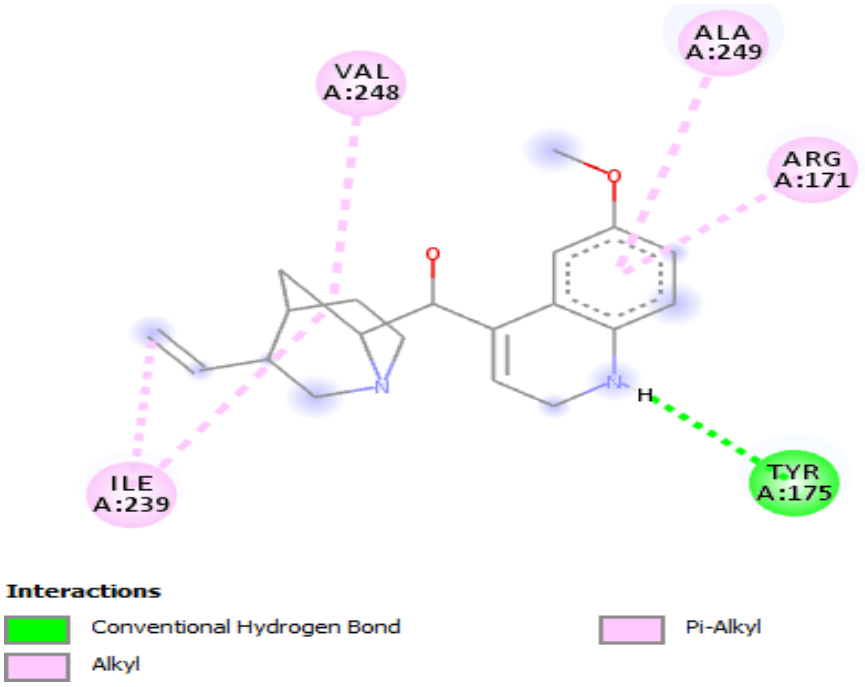
Chq: Chloroquine, UCTRL: infected but untreated control; NCTRL: Normal uninfected Control, EAFC: Ethyl acetate fraction of the combined leaves. (n=3).

Table 8: Molecular Docking Analysis and Binding Affinity Scores of Bioactive Compounds in the Combined leaves extract (ADME)

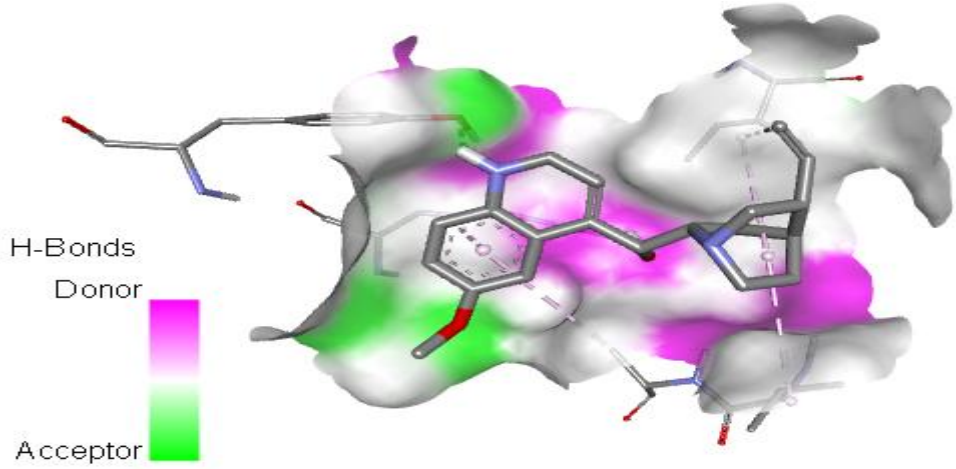
Ligand	Compound	MW	Binding Affinity	rmsd/ub	rmsd/lb
1oc4_prep_5	Cyclopropane	140	-2.5	0	0
1oc4_prep_47	Oxalic acid	342	-4.2	0	0
1oc4_prep_4	Pelargonic alcohol	144	-4.3	0	0
1oc4_prep_1	Decylene	140	-4.4	0	0
1oc4_prep_42	Pentadecanecarboxylic acid	256	-4.4	0	0
1oc4_prep_3	1-Nonanol	144	-4.5	0	0
1oc4_prep_60	Octyl 10-undecenoate	296	-4.5	0	0
1oc4_prep_6	Undecene, (E)-	154	-4.6	0	0
1oc4_prep_22	Margarinic acid	270	-4.6	0	0
1oc4_prep_49	4-n-Propylheptadecane	282	-4.6	0	0

1oc4_prep_53	Z-9-Tetradecenal	210	-4.6	0	0
1oc4_prep_15	Myristic acid	228	-4.8	0	0
1oc4_prep_43	Hexadecanoic acid	256	-4.8	0	0
1oc4_prep_24	Methyl palmitoleate	268	-4.9	0	0
1oc4_prep_27	Nonadecanoic acid	298	-4.9	0	0
1oc4_prep_30	Eicosadienoic acid	322	-4.9	0	0
1oc4_prep_57	15-Tetracosenoic acid	380	-4.9	0	0
1oc4_prep_56	Methyl erucate	352	-4.9	0	0
1oc4_prep_62	Hexadecenal	238	-4.9	0	0
1oc4_prep_51	cis-13-Docosenoic acid	338	-5	0	0
1oc4_prep_55	Brassidic acid	338	-5	0	0
1oc4_prep_12	Decanoic acid	186	-5.1	0	0
1oc4_prep_14	Capric acid	186	-5.1	0	0
1oc4_prep_34	Heneicosanoic acid	340	-5.2	0	0
1oc4_prep_35	Oleic Acid	282	-5.2	0	0
1oc4_prep_46	Hexadecanohydrazide	270	-5.2	0	0
1oc4_prep_54	Z-9-Tetradecenol	210	-5.2	0	0
1oc4_prep_23	Margaric acid	270	-5.3	0	0
1oc4_prep_25	Cyclopropaneoctanoic acid	282	-5.4	0	0
1oc4_prep_31	Elaidic acid	296	-5.4	0	0
1oc4_prep_59	(Z)-Methyl nervonate	380	-5.4	0	0
1oc4_prep_28	Pentadecanoic acid	242	-5.5	0	0
1oc4_prep_63	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	268	-5.5	0	0
1oc4_prep_7	alpha. -Farnesene	204	-5.6	0	0
1oc4_prep_9	Myristic acid	242	-5.6	0	0
1oc4_prep_17	Palmitic acid	256	-5.6	0	0
1oc4_prep_45	1-Pentadecanecarboxylic acid	256	-5.6	0	0
1oc4_prep_16	1-Tridecanecarboxylic acid	228	-5.7	0	0
1oc4_prep_18	Pentadecanecarboxylic acid	256	-5.7	0	0
1oc4_prep_19	Stearic acid	284	-5.8	0	0
1oc4_prep_29	Linolelaidic acid	294	-5.9	0	0
1oc4_prep_36	Erucic acid	338	-5.9	0	0
1oc4_prep_Chloroquine	Chloroquine	-	-6.1	0	0
1oc4_prep_Quininee	Quinine	-	-6.6	0	0

MW- Molecular Weight rmsd/ub- Root Mean Square Deviation/Upper Bound rmsd/lb- Root Mean Square Deviation/Lower Bound 1oc4_prep- = PDB Code used (Protein Data Bank Code).



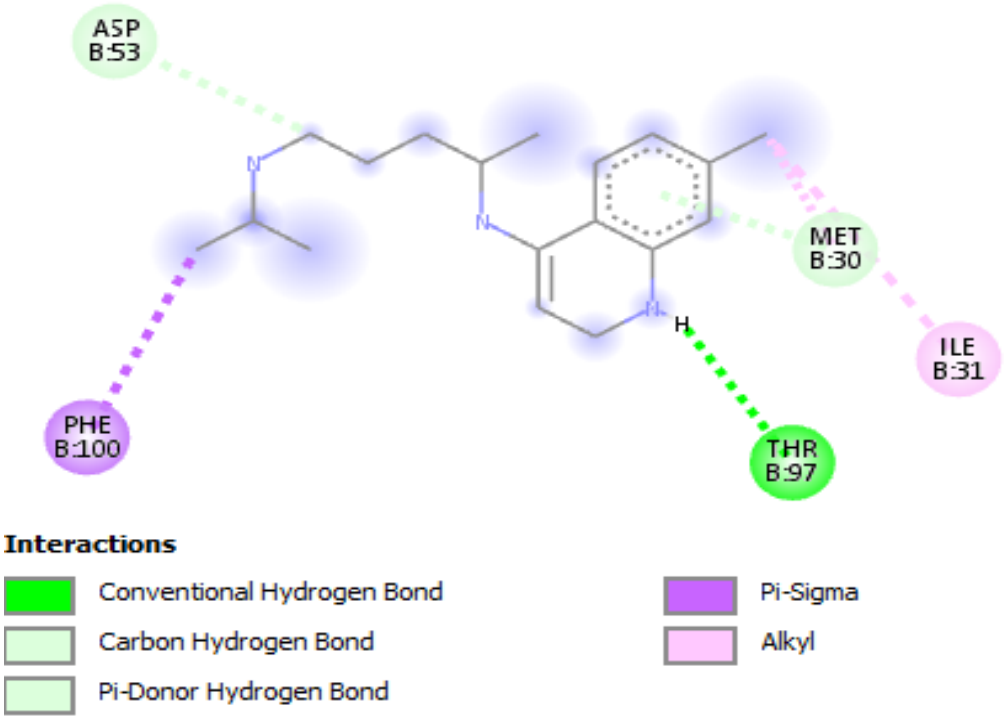
a: 2D residual Interactions



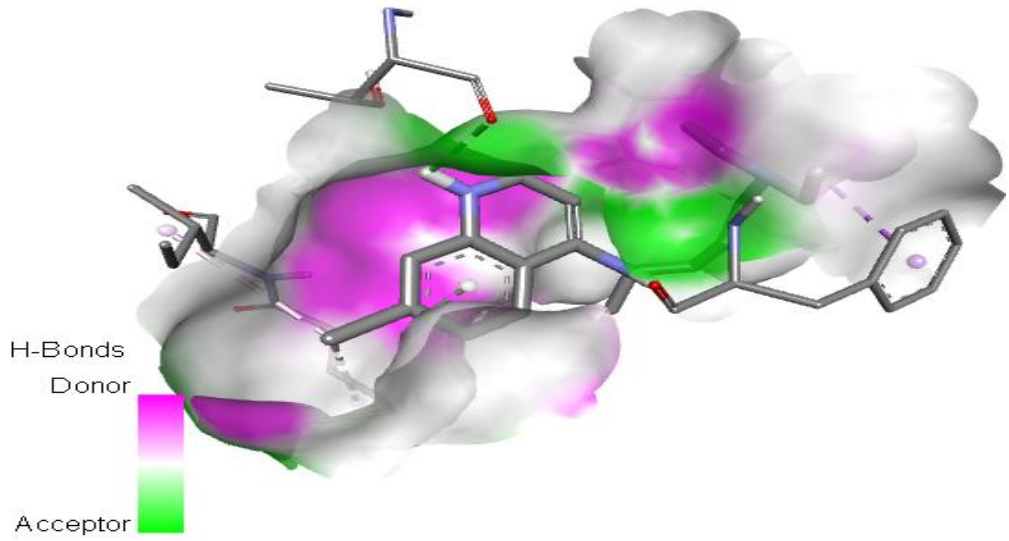
b: 3D H-bond surfaces

Figure 2 a, b: Binding pose view of quinine **(PDB: 1OC4)** with the targeted *Pb*LDH receptor.

a: 2D residual Interactions, **b**: 3D H-bond surfaces, **c**: 3D H-bond surfaces view; Targeted protein is depicted in the surface view, and the ligand compound is represented as a stick in the binding pocket.

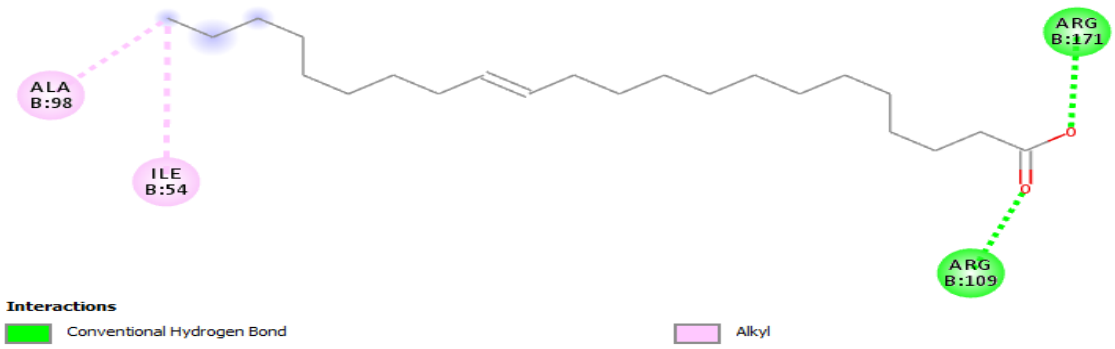


a. 2D residual Interactions

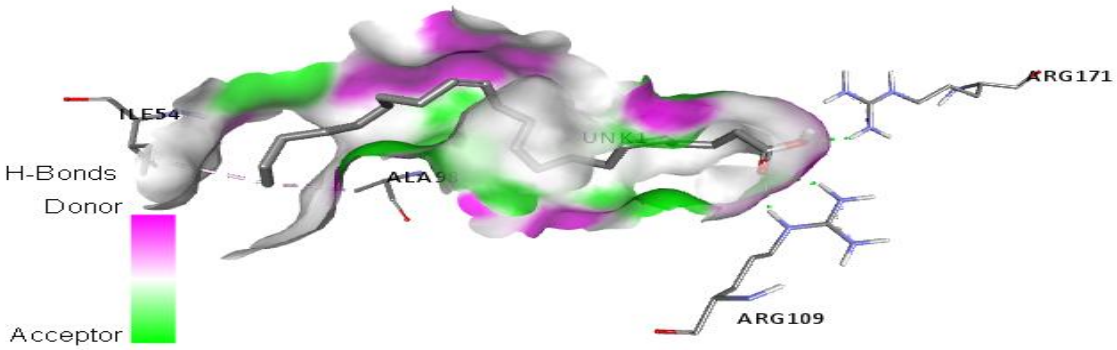


b. 3D H-bond surfaces

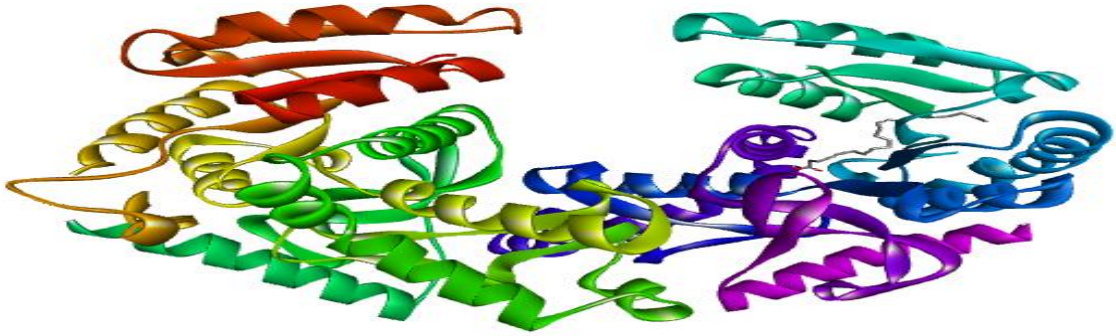
Figure 3 a, b: Binding pose view of Chloroquine **(PDB: 1OC4)** with the targeted *Pb*LDH receptor; **a**: 2D residual interactions, **b**: 3D H-bond surfaces, **c**: 3D H-bond surface view Targeted The protein is depicted in the surface view, and the ligand compound is represented as a stick in the binding pocket.



a: 2D residual Interactions

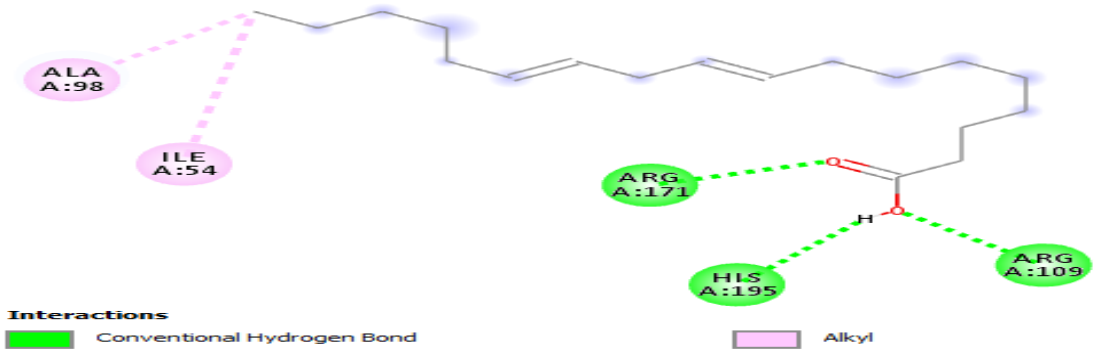


b: 3D H-bond surfaces

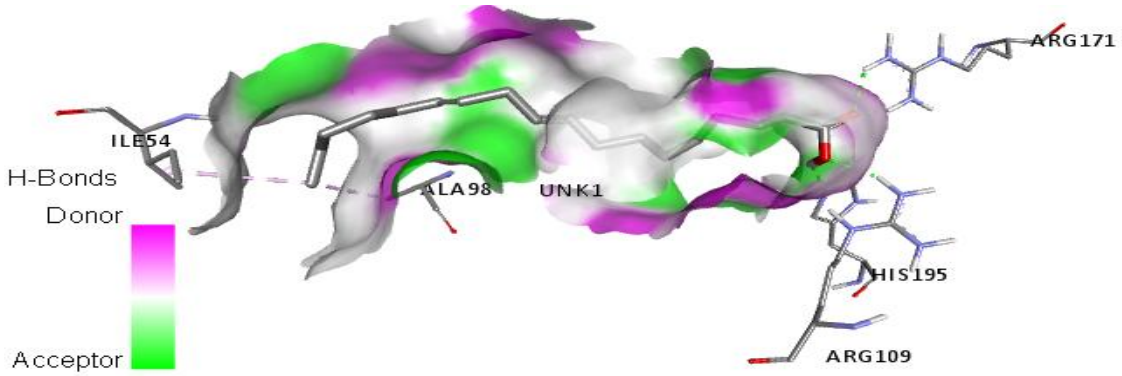


c: 3D H-bond surfaces

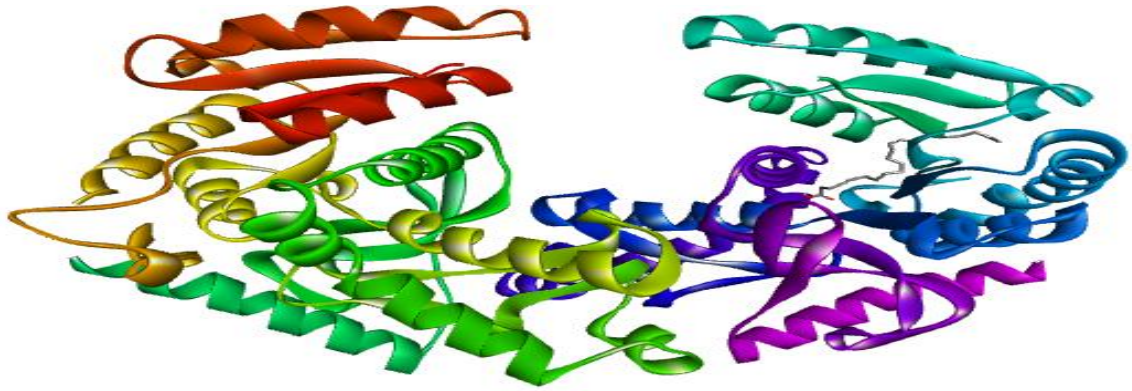
Figure 4 a, b, c: Binding pose view of COMPLEX 36 (PDB: 1OC4) (Erucic acid) with the targeted *Pb*LDH receptor; **a**: 2D residual interactions, **b**: 3D H-bond surfaces, **c**: 3D H-bond surface view Targeted protein is depicted in the surface view and the ligand compound as the stick in the binding pocket.



a: 2D residual Interactions



b: 3D H-bond surfaces



c: COMPLEX 29 (PDB: 1OC4)

Figure 5 a, b, c: Binding pose view of of COMPLEX 29 (PDB: 1OC4) (Linolelaidic acid) with the targeted *Pb*LDH receptor; **a**: 2D residual Interactions, **b**: 3D H-bond surfaces, **c**: Targeted protein is depicted in surface view and ligand compounds as sticks in the binding pocket.

Discussion

GC-MS analysis identified several bioactive compounds, including fatty acids, methyl esters, and volatile organic substances, in both *D. oliveri* and *M. lucida* extracts. The presence of these compounds suggests significant pharmacological potential, particularly in terms of lipid-based bioactivity. GC-MS is effective for separating and identifying compounds based on their mass-to-charge ratio (m/z), retention index, and molecular weight, confirming the presence of bioactive constituents with potential medicinal applications (Reineccius *et al*., 2024; Wakoli *et al*., 2024). This is in agreement with a previous report (Ranjan Maji *et al*., 2023), which stated that the combination of GC and MS allows for the identification of compounds by comparing their retention times and mass spectra with those of known standards or library databases. *D. oliveri* contains notable fatty acids and methyl esters, such as methyl myristate, linoleic acid, oleic acid, and palmitoleic acid, all of which have pharmacological significance (Yakubu *et al*., 2017). Similarly, *M. lucida* contained bioactive compounds including phthalic acid, benzenedicarboxylic acid, myristic acid, and decanoic acid. These findings support existing reports on the medicinal potential of these plants, particularly their anti-inflammatory and antimicrobial properties (Adekunle *et al*., 2023).

The combined plant extract subjected to GC-MS analysis revealed more diverse bioactive compounds than those in the individual plant extracts. This supports the hypothesis that the synergy between plant constituents enhances the range and potency of bioactive compounds (Chaachouay, 2025). Reports have also indicated that synergistic effects among phytochemicals can significantly improve their biological activities (Nock and Amlabu, 2020).

The therapeutic effects of medicinal plants stem from the activities of their bioactive compounds. Nock and Amlabu, (2020) reported that, dodecanoic acid, methyl ester; carotol; hexadecanoic acid, methyl ester; 9-octadecenoic acid (Z), methyl ester (oleic acid); methyl stearate; heptadecanoic acid, 16-methyl-, methyl ester, possessed antimalarial activities. The high concentrations of these compounds in *D. oliveri* are consistent with reports demonstrating its potent antiplasmodial activity (Mba *et al*., 2022; Oladeji *et al*., 2022). Understanding the specific bioactive compounds responsible for antimalarial activity is crucial for future research. Studies suggest that the synergy of phytochemicals in plant combinations can enhance antimicrobial efficacy (Vaou *et al*., 2022). The methanol fraction (MFC) was the most potent of all the extracts and fractions used. This may be due to the solvent polarity and bioactive compound extraction. This is consistent with the report of Lee *et al*. (2024) that methanol is a polar solvent, which may have extracted more polar bioactive compounds, such as alkaloids and glycosides, that are more soluble in polar solvents, leading to a more potent antiplasmodial fraction. Methanol may have stabilized the bioactive compounds, prevented their degradation or oxidation, and improved their solubility (Ojatula, 2021), thereby making the bioactive compounds in MFC more available for antiplasmodial activity.

Molecular docking analysis is necessary to identify the actual bioactive compounds responsible for antiplasmodial activity. Simulating the antimalarial potential of these metabolites using molecular docking is essential to determine the activities of these bioactive compounds. Targeting the *P. berghei* lactate dehydrogenase *(Pb*LDH) receptor has been shown to be vital, efficient, and effective in mimicking the antimalarial potential of the aqueous crude extracts and fractions of the combined leaf extract as antimalarial drug candidates (Aykul and Martinez-Hackert, 2016).

Lactate dehydrogenase is essential for the anaerobic conditions of *Plasmodium* species, which may be destroyed by the Plasmodium parasite when targeted by any bioactive compound. This corroborates the report by Enenebeaku *et al.* (2021) that inhibiting lactate dehydrogenase disrupts the synthesis of lactate from pyruvate, alters energy production in the parasites, and may destroy the parasites.

The molecules identified showed good binding affinity scores with the active site of the *Pb*LDH receptor, ranging between (−6.6 and − 4.2 kcal/mol) in comparison with quinine and chloroquine as standard references. The relatively high and close binding affinity (kcal/mol) of quinine, chloroquine, erucic acid, and linolelaidic acid may be due to their similar bioavailability and high rate of gastrointestinal absorption. Abdullahi *et al*., (2021) positions that, drug-likeness and pharmacokinetic profile prediction results for the selected ligands with higher binding affinities show zero violations of Lipinski rules with similar bioavailability, and high rate in gastrointestinal absorption.

The number of hydrogen bonds and other interactions are crucial in influencing the binding affinity and interaction strengths of the stable ligand-receptor complexes formed (Yakubu *et al*., 2025; Karou *et al*., 2011; Rasoanaivo *et al*., 2011). This provides structural insight into why quinine, chloroquine, erucic acid, and linolelaidic acid were able to bind to *the Pb*LDH active pocket and agrees with the explanation of Yakubu *et al*. (2025) that the number of hydrogen bonds and other interactions are crucial in influencing the binding affinity and interaction strengths of stable ligand-receptor complexes.

Conclusion

GC-MS analysis revealed 65 bioactive compounds in the combined aqueous extract of *D. oliveri* and *M. lucida* leaves. The antiplasmodial activity of the aqueous crude extracts and fractions was highest in the methanol fraction. Molecular docking studies showed that erucic and

linolelaidic acids are the two bioactive compounds with the highest binding affinity for the targeted *Pb*LDH receptor, indicating the highest antiplasmodial activities of the two compounds.

Conflicts of interest

The authors declare no conflicts of interest. The content of this manuscript for publication is in partial fulfilment of the award of a higher degree in NDA.

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