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Molecular Docking Interactions of *P. falciparum* Drug Target Receptors with Bioactive Compounds of *Jatropha tanjorensis* Leaf Extract

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ABSTRACT

The growing challenge of antimalarial drug resistance underscores the need to explore novel therapeutic agents derived from plant sources. Molecular docking studies were conducted to ascertain the potential mode of action of the bioactive compounds in *Jatropha tanjorensis* leaf extract. This study investigatesd the *in silico* molecular docking interactions between key *Plasmodium falciparum* drug target receptors and the bioactive compounds identified in a fraction of ethyl acetate extract of *Jatropha tanjorensis* leaves. Eighteen bioactive compounds were identified through gas chromatography-mass spectrometry (GC-MS) analysis. Molecular docking was then performed on the identified compounds with sixteen *P. falciparum* target receptors to predict the molecular interactions *in silico*. The results revealed binding affinities of receptor-ligand interactions ranging from -4.1 to -11.1 kcal/mol. Notably, among these bioactive compounds, phthalic acid, 5-methylhex-2-yl butyl ester; 1,1'-bicyclohexyl, 2-(2-methylpropyl)-, trans-; 9,12-octadecadienoic acid methyl ester; phytol; squalene; and heneicosane, 11-pentyl-, exhibited significant and consistent receptor-ligand interactions with binding affinities ranging from -7.0 to -11.1 kcal/mol. Squalene, in particular, demonstrated interactions with multiple target receptors, suggesting its potential to overcome drug resistance. These findings underscore the inhibitory potential of bioactive compounds from *Jatropha tanjorensis* leaves as promising candidates for novel antimalarial agents, warranting further investigation and development.

Keywords: Molecular Docking, *Plasmodium falciparum*, *Jatropha tanjorensis*, Bioactive Compounds.

INTRODUCTION

Protozoan parasites of the genus *Plasmodium* cause malaria, which is one of the most deadly infectious diseases in the world [1, 2, 3]. *Plasmodium falciparum* is the most lethal species, accounting for most malaria-related deaths [4, 5]. The ongoing emergence of drug-resistant strains of *P. falciparum* has made current treatment strategies extremely difficult, highlighting the urgent need for novel antimalarial agents. The World Health Organization (WHO) reported 247 million malaria cases and 619,000 related deaths in 2021, with 95% of cases and 96% of deaths occurring in the WHO African region. Nigeria accounted for 31.3% of the global malaria deaths and 27% of all malaria cases during that period [2].

Jatropha tanjorensis, a perennial plant from the Euphorbiaceae family, has shown promising antimalarial properties in preliminary studies against *P. falciparum* and *P. berghei* [4, 5, 6]. The plant is traditionally used in various cultures for its medicinal properties, potential as a blood-replenishing agent, and other therapeutic benefits [7]. High amounts of antioxidants can be found in *J. tanjorensis* leaves. Through their ability to scavenge free radicals, these substances shield cells from oxidative stress and lower the chance of developing chronic illnesses including cancer and heart disease [8]. Extracts from the leaves of *J. tanjorensis* have shown strong anti-inflammatory effects. These benefits include lowering pain and swelling and helping to manage inflammatory diseases [9].

Antimicrobial action against a variety of diseases has been demonstrated by *J. tanjorensis* leaves. For treating diseases, this makes the plant a possible source of natural antimicrobial drugs [10]. Extracts from the leaves of *J. tanjorensis* have been shown to shield the liver against toxicity-induced harm. Bioactive substances including flavonoids and saponins are responsible for this hepatoprotective effect [11]. Leaf extracts may also benefit diabetic individuals, as studies have shown they can help control blood glucose levels. Improved glucose absorption and increased insulin secretion are linked to the plant's hypoglycemic impact [12]. Despite these applications, its complete pharmacological potential is still unknown, especially concerning its bioactive components and how they work to prevent malaria. This study aims to elucidate the molecular docking interactions of *P. falciparum* drug target receptors with bioactive compounds of *Jatropha tanjorensis* leaf extract.

MATERIALS AND METHODS

Extraction and fractionation of plant material

The plant material was prepared by air drying it and grinding it into a powder [13]. The plant powder was extracted using the maceration technique. The dried powder was soaked in ethyl acetate for 72 hours, then filtered and concentrated [14]. The resulting extract was fractionated using thin-layer chromatography and gel column chromatography. Thin-layer chromatography was performed on the extract using aluminum foil coated with silica gel [15], while column chromatography was performed using silica gel-60 and n-hexane and ethyl acetate. The best solvent system for separation was 8:2 (n-hexane: ethyl acetate) equivalent to 4:1 (n-hexane: ethyl acetate). The column fractions were labeled and monitored using TLC plates [16].

Gas chromatography and mass spectrometry analysis

A fraction of the ethyl acetate extract of *J. tanjorensis* leaf was analyzed using GC-MS to identify bioactive compounds and their masses. The fraction was dissolved in n-hexane, separated into molecules, and ionized via mass spectrometer. The data was used to determine the chemical names, molecular structures, and masses of the compounds. This was achieved by comparing their retention times and masses with those of reference compounds in the reference library. The analysis was conducted using a GC-MS Clarus 500 Perkin Elmer system following specific conditions outlined by Bharathy and Uthayakumari [17]. The specific conditions included using an Elite-1 fused silica capillary column, helium as the carrier gas, and oven temperatures starting at 110 °C (isothermal for 2 minutes), followed by an increase of 10 °C/min to 200 °C, then 5 °C/min to 280 °C, and finally held isothermally at 280 °C for 9 minutes. The GC running time was 36 minutes.

In silico receptor-ligand interaction

The *In-silico* receptor-ligand interaction was evaluated according to the methods described by Emmanuel *et al.* [18]. The best pose with the lowest energy binding affinity between receptor-ligand interaction was determined using softwares that includes Chem Draw Ultra 12.0, Spartan 14, UCSF Chimera, MG tools, AutoDock vina, Discovery Studio 2020, Cygwin Terminal. The protein data bank (PDB) was used to prepare the PDB structure of important antimalarial target receptors of *Plasmodium falciparum.* Ligand structure preparation involved converting 2D structures of ligands to 3D structures and optimizing them using MGL Tools and Chimera UCSF.

The grid box module in AutoDock tools was used to determine the configurations of the targets' active sites. Docking tests were performed using Auto Dock Vina software to determine the binding conformation and interactions that underlie the activity of the compounds. The docking process for the receptors was validated before docking the test compounds by separating the co-crystallized ligand from the receptor's crystal structure obtained from the protein data bank and re-docking using the set-up parameters. The docking and binding analysis were aimed at observing the binding of compounds to the active site and allosteric site of receptors. The bioactive compounds were re-docked using virtual screening software (Auto Dock Vina) and visualized using the Discovery Studio 2020 client [19].

RESULTS AND DISCUSSION

The results in Table 1 show the Gas Chromatography and Mass Spectroscopic (GC-MS) analysis of compounds in the column fraction of ethyl acetate extract of *J. tanjorensis* leaf.

Table 1: Gas chromatography and mass spectroscopic (GC-MS) analysis of compounds in column fraction of ethyl acetate extract of *J. tanjorensis* leaf

Peak No.	Retention Time	Peak Area	Compound Name	Probability Quality (%)	Ligand ID
1	13.625	4.66	Butylated Hydroxytoluene	98	Lig0
2	15.386	0.14	2-Tetradecene, (E)-	93	Lig1
3	17.692	0.27	Dodecanoic acid, propyl ester	99	Lig2
4	19.884	0.31	9-Eicosene, (E)-	95	Lig3
5	21.984	0.24	Tetradecanoic acid, propyl ester	98	Lig4
6	22.739	0.56	Hexadecanoic acid, methyl ester	97	Lig5
7	23.554	0.43	Phthalic acid, 5-methylhex-2-yl butyl ester	90	Lig6
8	24.002	0.55	1-Octadecene	99	Lig7
9	24.091	0.43	Hexadecanoic acid, ethyl ester	84	Lig8
10	25.444	0.52	1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-	60	Lig9
11	25.642	0.43	2-Octyldodecyl butyrate	49	Lig10
12	25.927	1.17	Hexadecanoic acid, propyl ester	99	Lig11
13	25.997	1.41	9,12-Octadecadienoic acid, methyl ester	99	Lig12
14	26.115	3.86	12-Octadecenoic acid, methyl ester	99	Lig13

15	26.399	0.99	Phytol	91	Lig14
16	27.345	0.26	13-Octadecenal, (Z)-	78	Lig15
17	36.104	47.21	Hentriacontane	98	Lig16
18	36.399	47.21	Hentriacontane	98	Lig16
19	37.820	0.35	Squalene	95	Lig17
20	38.338	1.82	Heneicosane, 11-pentyl-	96	Lig18

The results identified twenty phytoconstituents with retention times ranging from 15.386 to 38.338. The peak areas ranged from 0.14 to 47.2, and the probability percentages of most of the compounds fell within the 90 to 99% range. Only four compounds had probability percentages below 90%.

Figure 1 presents the chromatogram of compounds identified in column fraction by GC/MS analysis.

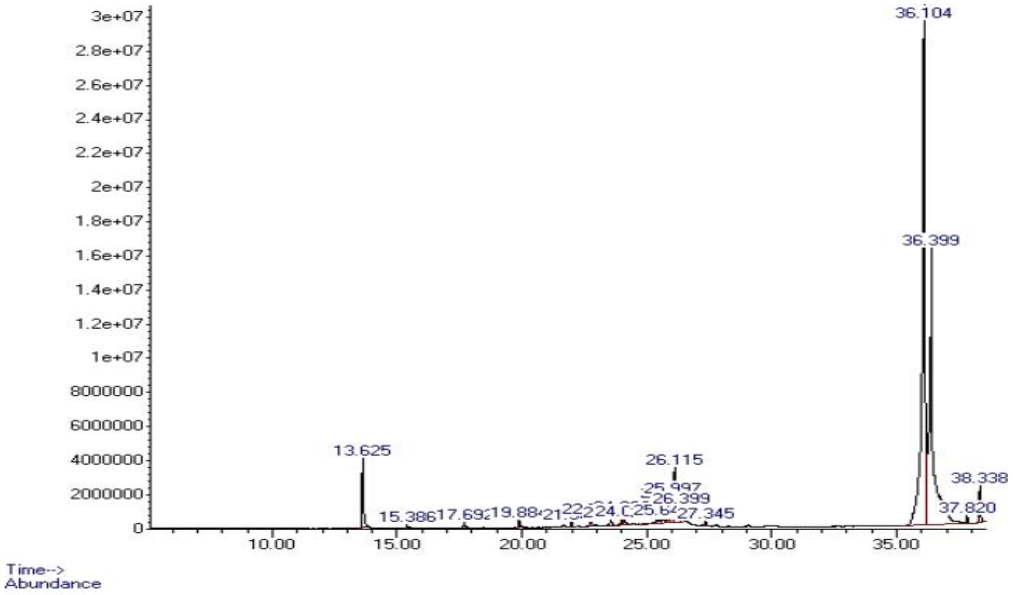


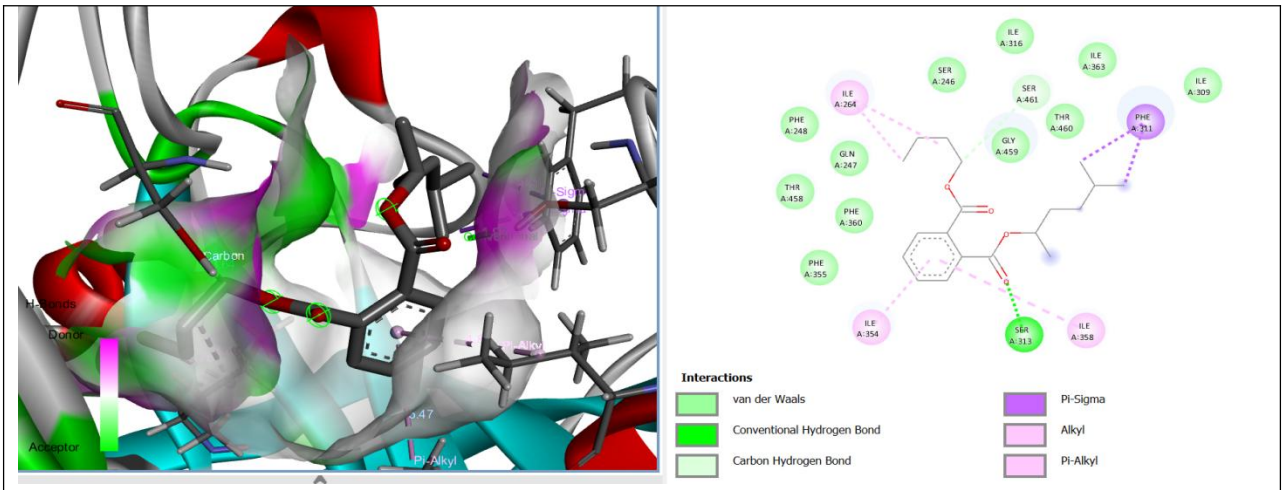
Figure 1: GC-MS chromatogram of compounds in column fraction of ethyl acetate extract of *J. tanjorensis* leaf

Table 2 presents the ligands displaying the least binding affinity to the receptors (enzymes and proteins) of *P. falciparum*.

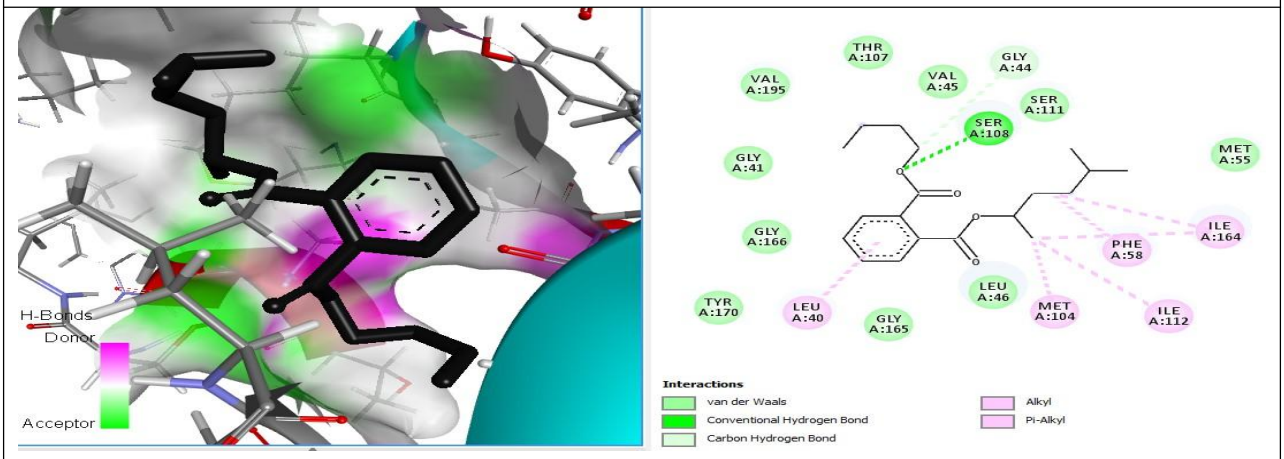
These compounds were utilized for molecular interactions using the Discovery Studio Visualizer. The binding affinities of the bioactive compounds ranged from -4.7 to -11.1 kcal/mol.

Although, the binding energy observed in receptor-ligand interactions can vary based on factors such as the 3D shape of the protein, the types of amino acids present in the active site, and their respective positions, distinct binding affinity profiles were noted in each of the receptor-ligand complexes analyzed in this investigation.

Figure 2 shows the receptor-ligand interactions between Phthalic acid, 5-methylhex-2-yl butyl ester and PlasmepsinX (-7.1 kcal/mol), Plasmodium falciparum Dihydrofolate Reductase (-7.1 kcal/mol), and Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.1 kcal/mol). The interaction with PlasmepsinX involved several amino acid residues which include ILE 264, GLY 459, SER 461, PHE 311, ILE 358, SER 313, and ILE 354 using Van der Waals, Conventional hydrogen bond, Carbon-hydrogen bond, Pi-sigma,

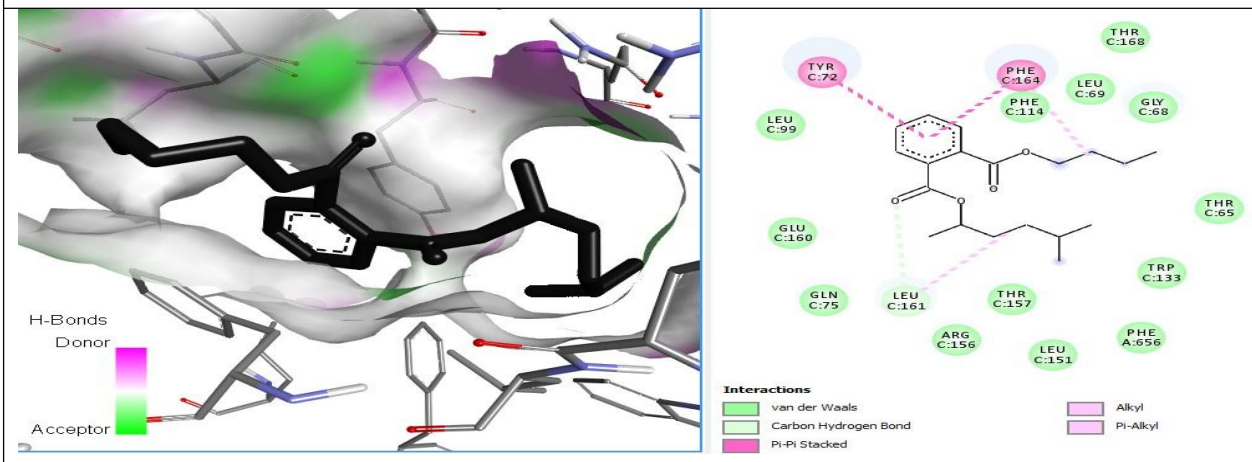


3D and 2D Interactions of Phthalic acid, 5-methylhex-2-yl butyl ester and Plasmepsin X (-7.1 kcal/mol)



3D and 2D Interactions of Phthalic acid, 5-methylhex-2-yl butyl ester and Plasmodium

falciparum Dihydrofolate Reductase (-7.1 kcal/mol)



3D and 2D Interactions of Phthalic acid, 5-methylhex-2-yl butyl ester and Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.1 kcal/mol)

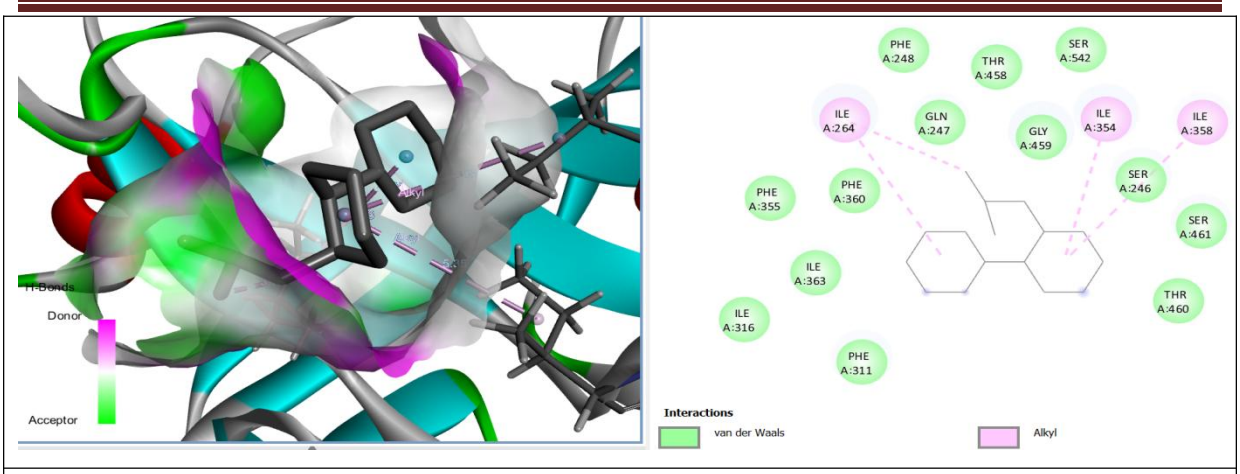
Figure 2: Molecular Interactions of Phthalic acid, 5-methylhex-2-yl butyl ester with some Receptors of *P. falciparum*

Alkyl and pi-Alkyl. On the other hand, the interaction with Plasmodium falciparum Dihydrofolate Reductase involved GLY 44, SER 108, LEU 40, LEU 46, MET 104, ILE 112, PHE 58, and ILE 164 using Van der Waals, Conventional hydrogen bond, Carbon-hydrogen bond, Alkyl and pi-Alkyl. while the interaction with Plasmodium falciparum Erythrocyte Membrane Protein 1 involved TYR 72, PHE 164, PHE 114, LEU 161, and GLY 68 using Van der Waals, Carbon-hydrogen bond, Pi-pi stacked, Alkyl and pi-Alkyl.

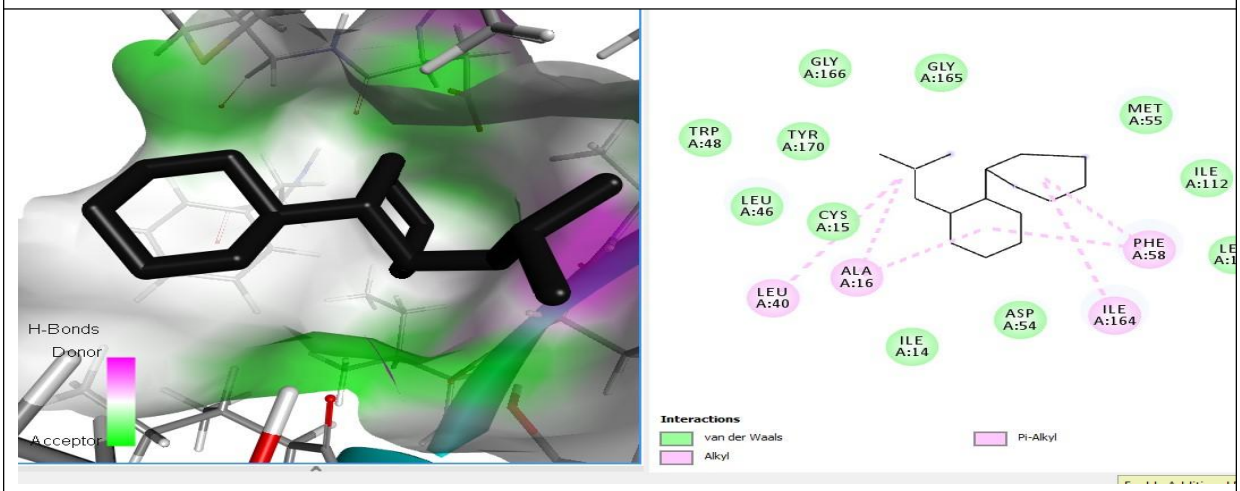
Table 2: Ligands with Lowest Binding Affinity to the Receptors (Enzymes and Proteins) of *P. falciparum.*

Ligand ID	Compound Name	FP 2	FP 3	PL II	PLI V	PL X	SE RAs	PfD HFR	PfL DH	PfC DPK1	GAP DH	PfE MP1	AMA 1	PfM DR1
Lig0	Native Ligand	-5.4	-8.5	-8.0	-8.5	-11.8	-2.3	-12.0	-6.7	-11.7	-10.6	-8.8	-4.6	-8.2
Lig6	Phthalic acid, 5-methylhex-2-yl butyl ester	-6.6	-6.7	-6.1	-6.6	-7.1	-5.3	-7.1	-5.2	-6.4	-5.9	-8.1	-6.5	-6.4
Lig9	1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-	-6.0	-6.6	-6.1	-6.6	-7.3	-5.6	-7.3	-6	-6.4	-6.4	-8.9	-6.5	-7.2
Lig12	9,12-Octadecadienoic acid, methyl ester	-4.7	-5.6	-4.8	-6.0	-6.4	-4.2	-6.1	-4.9	-5.4	-5.3	-8.0	-6.0	-5.2
Lig14	Phytol	-5.2	-5.5	-5.7	-6.3	-6.9	-4.9	-6.3	-5.6	-5.9	-5.6	-8.3	-6.2	-5.6
Lig17	Squalene	-7.0	-7.3	-6.5	-7.1	-7.8	-5.1	-8.2	-6.2	-7.1	-7.9	-11.1	-6.7	-6.5
Lig18	Heneicosane, 11-pentyl-	-4.4	-5.7	5.5	-5.6	-5.6	-4.1	-6.2	-4.4	-5.6	-4.9	-8.1	-6.1	-5.2

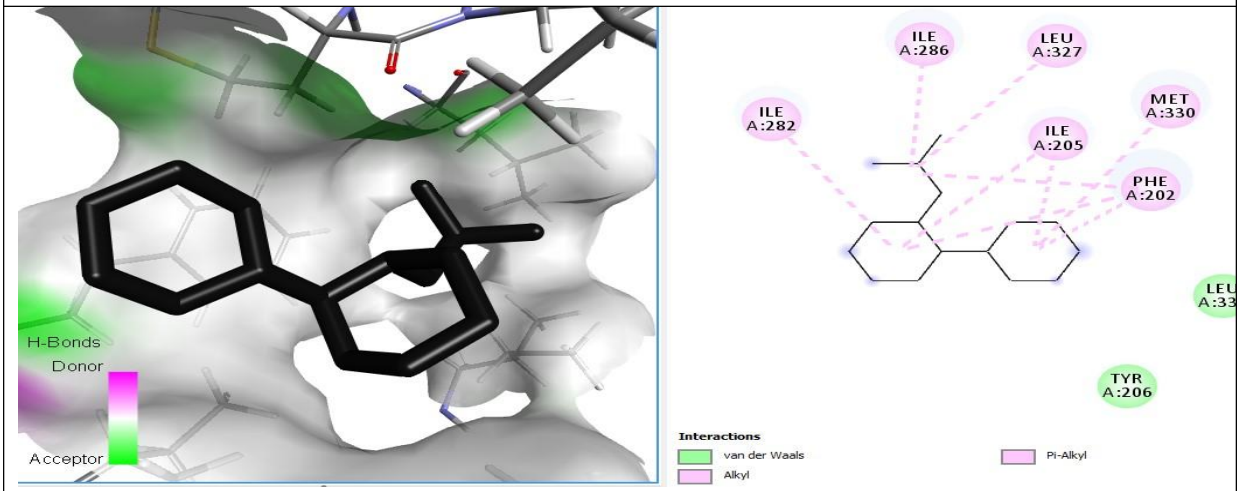
Source: Auto Dock Vina, 2010 (Software Application) Key: FP2 = Falcipain 2**; FP3** = Falcipain 3; **PLII** = Plasmepsin II; **PLIV =** Plasmepsin IV; **PLX** = Plasmepsin X; **SERAs =** Serine Repeat Antigens; **PfDHFR** = Plasmodium falciparum Dihydrofolate Reductase; **PfLDH** = Plasmodium falciparum Lactate Dehydrogenase, **PfCDPK1 =** Plasmodium falciparum Calcium-Dependent Protein Kinase 1, **GAPDH=** glyceraldehyde-3-phosphate dehydrogenase, **PfEMP1** = Plasmodium falciparum Erythrocyte Membrane Protein 1**; AMA1** = Apical Membrane Antigen 1; **PfMDR1** = Plasmodium falciparum Multidrug Resistant Protein 1.



3D and 2D Interactions of 1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer and PlasmepsinX (-7.3 kcal/mol)



3D and 2D Interactions of 1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer and Plasmodium falciparum Dihydrofolate Reductase (-7.3 kcal/mol)

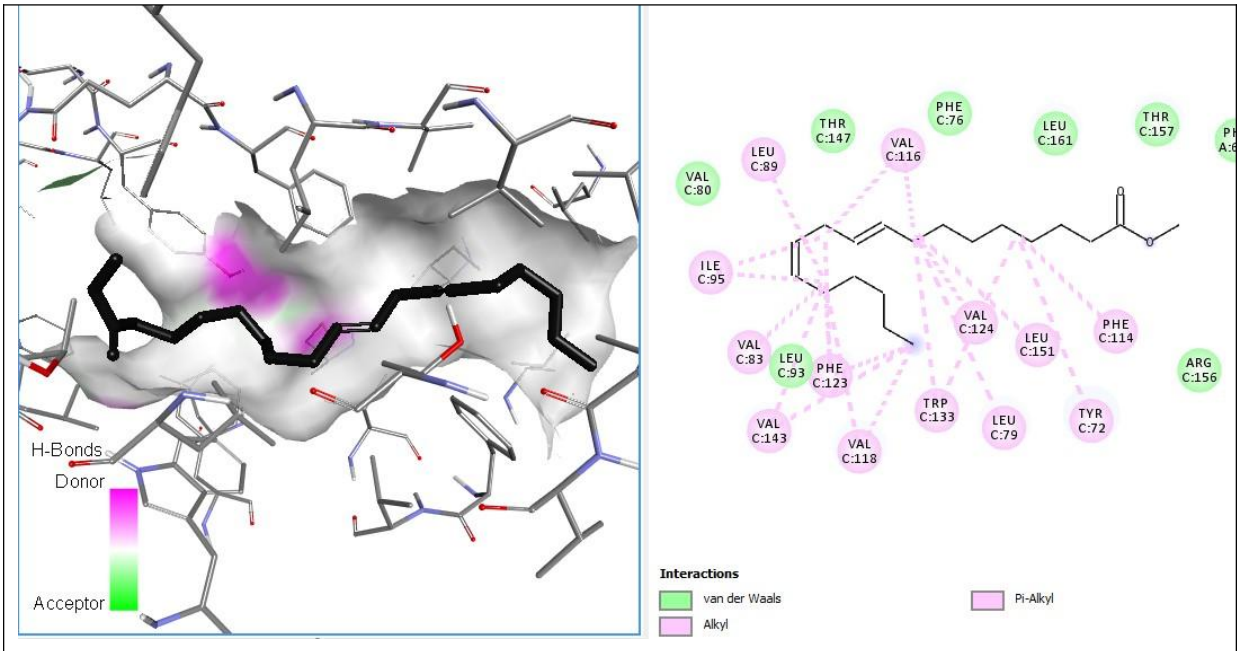


3D and 2D Interactions of 1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer and Plasmodium falciparum multidrug resistant Protein 1 (-7.2 kcal/mol)

Figure 3: Molecular Interactions of 1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer with some Receptors of *P. falciparum*

Figure 3 shows the receptor-ligand interactions between 1,1'-Bicyclohexyl, 2-(2-methylpropyl), trans-isomer and PlasmepsinX (-7.3 kcal/mol), Plasmodium falciparum Dihydrofolate Reductase (-7.3 kcal/mol), and Plasmodium falciparum multidrug resistant Protein 1 (-7.2 kcal/mol). The interaction with PlasmepsinX involved several amino acid residues which include ILE 264, PHE 360, GLN 247, GLY 459, ILE 354, SER 246, and ILE 358 using Van der Waals, and Alkyl bond. On the other hand, the interaction with Plasmodium falciparum Dihydrofolate Reductase involved LEU 40, ALA 16, PHE 58, ILE 164, CYS 15, ASP 54, ILE 14, and MET 55 using Van der Waals, Alkyl, and Pi-Alkyl bond. While the interaction with Plasmodium falciparum multidrug-resistant Protein 1 involved ILE 282, ILE 286, LEU 327, ILE 205, MET 330, PHE 202, LEU 333, and TYR 206 using Van der Waals, Alkyl, and Pi-Alkyl bond.

Figure 4 shows the receptor-ligand interactions between 9,12-Octadecadienoic acid, methyl ester (Linoleic acid) and Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.0 kcal/mol).

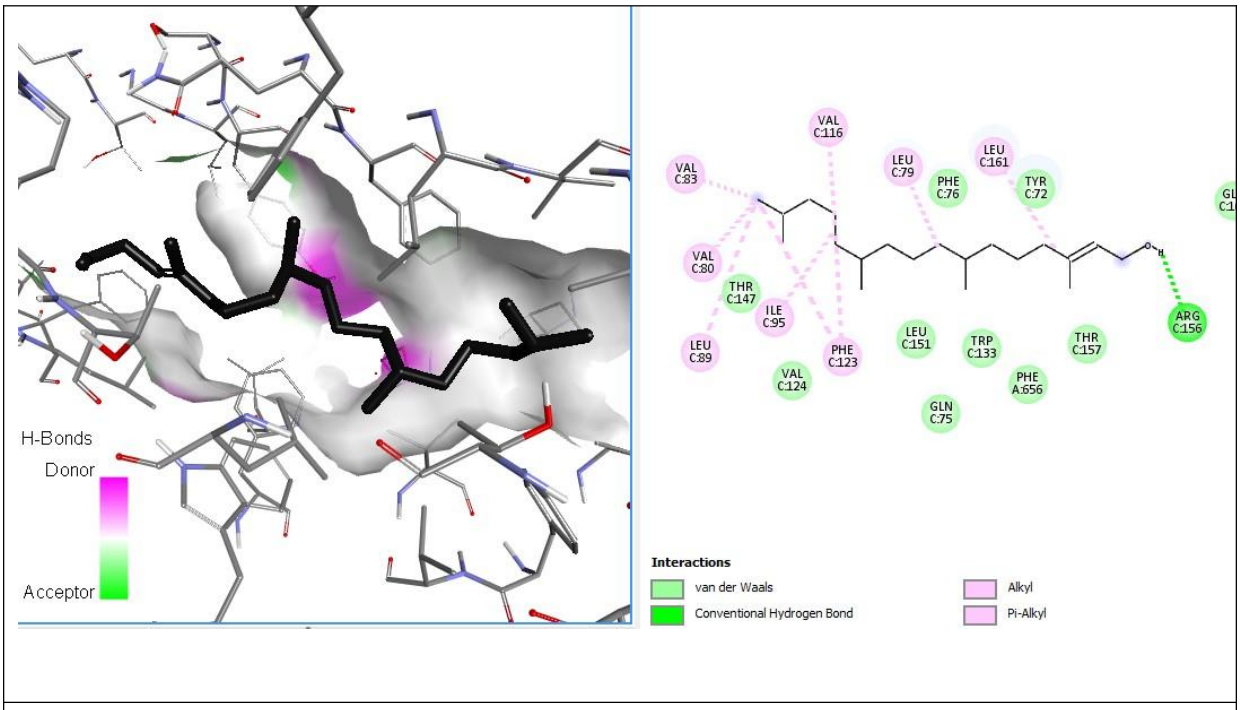


3D and 2D Interactions of 9,12-Octadecadienoic acid, methyl ester (Linoleic acid) and Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.0 kcal/mol)

Figure 4: Molecular Interactions of 9,12-Octadecadienoic acid, methyl ester (Linoleic acid) with a Receptor of *P. falciparum*

The interaction with Plasmodium falciparum Erythrocyte Membrane Protein 1 involved LEU 89, THR 147, VAL 80, VAL 116, ILE 95, VAL 83, LEU 93, PHE 123, VAL 143, VAL 118, TRP 133, LEU 79, VAL 124, LEU 151, TYR 72, PHE 114, ARG 156, PHE 76, LEU 161, and THR 157 using Van der Waals, Alkyl, and Pi-Alkyl bond.

Figure 5 shows the receptor-ligand interactions between Phytol and Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.3 kcal/mol). The interaction with Plasmodium falciparum .

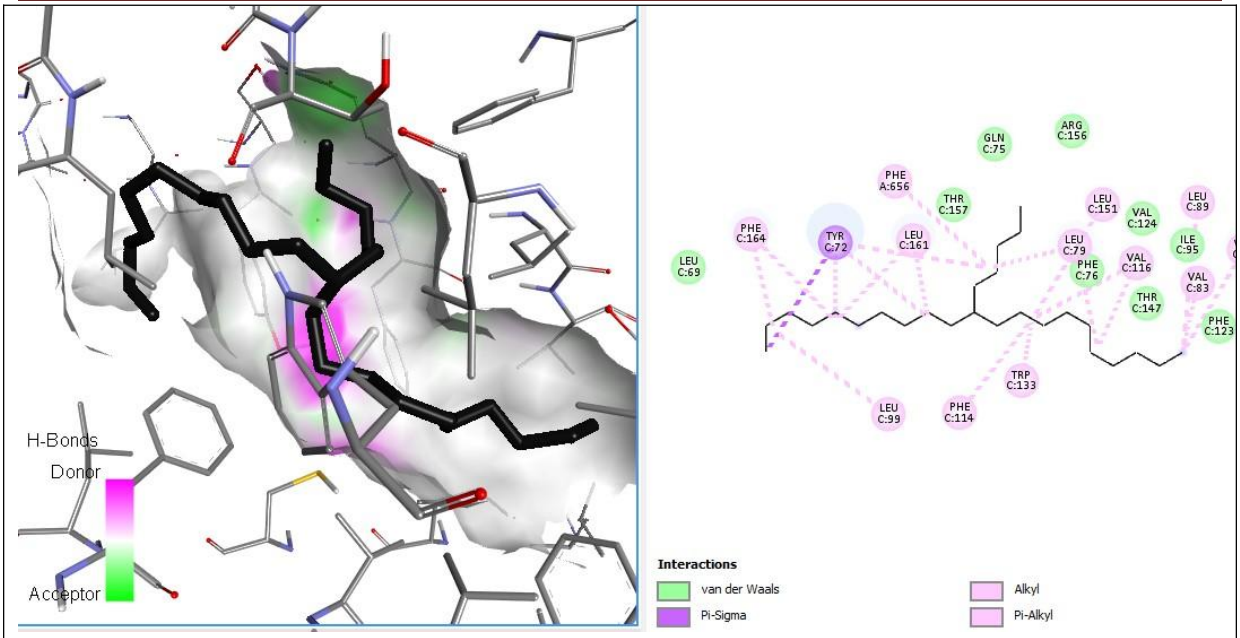


3D and 2D Interactions of Phytol and Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.3 kcal/mol)

Figure 5: Molecular Interactions of Phytol with a Receptor of *P. falciparum*

Erythrocyte Membrane Protein 1 involved VAL 83, VAL 80, LEU 89, THR 149, ILE 95, VAL 124, PHE 123, VAL 116, LEU 79, PHE 76, LEU 151, LEU 161, TYR 72, and ARG 156 using Van der Waals, Conventional hydrogen bond, Alkyl, and Pi-Alkyl bond.

Figure 6 shows the receptor-ligand interactions between Heneicosane, 11-pentyl- and *Plasmodium falciparum*

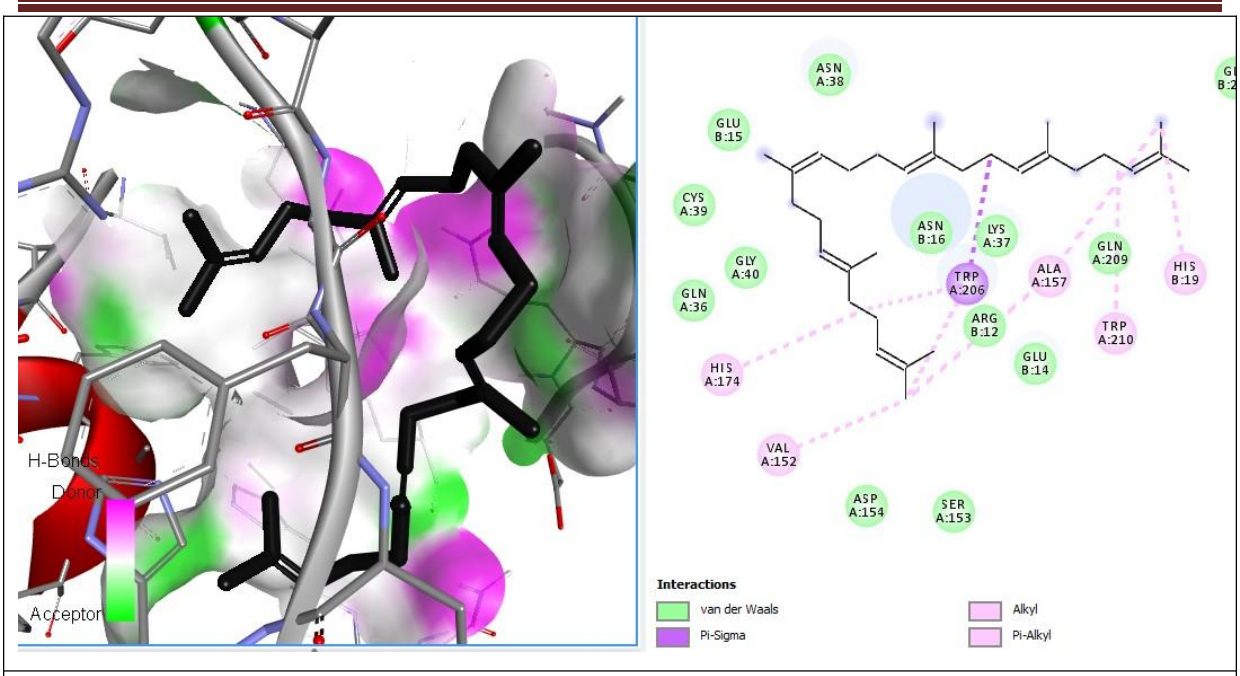


3D and 2D Interactions of Heneicosane, 11-pentyl- and Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.1 kcal/mol)

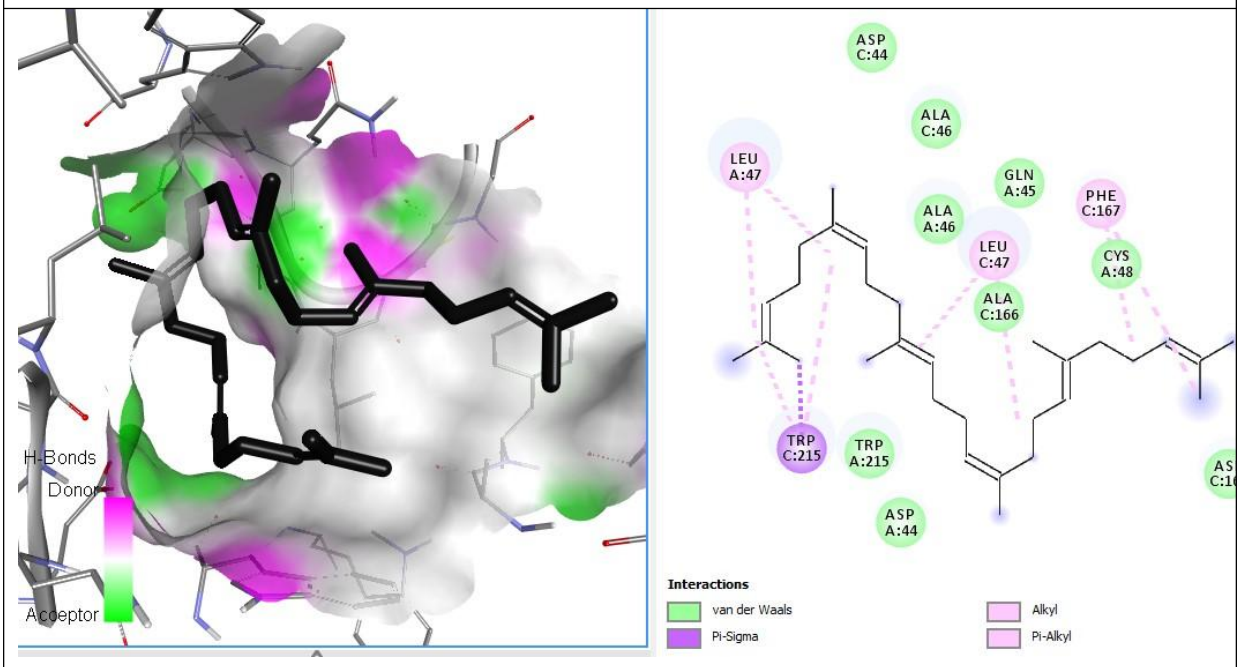
Figure 6: Molecular Interactions of Heneicosane, 11-pentyl- with a Receptor of *P. falciparum* erythrocyte membrane Protein 1 (-8.1 kcal/mol).

The interaction with *Plasmodium falciparum* Erythrocyte Membrane Protein 1 involved PHE 164, TYR 72, LEU 161, PHE 656, THR 157, LEU 99, PHE 114, TRP 133, LEU 79, LEU 89, PHE 76, VAL 116, THR 147, VAL 83, VAL 80, PHE 123, and VAL 124 using Van der Waals, Pi-sigma, Alkyl, and Pi-Alkyl bond.

Figures 7a-7d show the receptor-ligand interactions between Squalene, and Falcipain 2 (-7.0 kcal/mol),

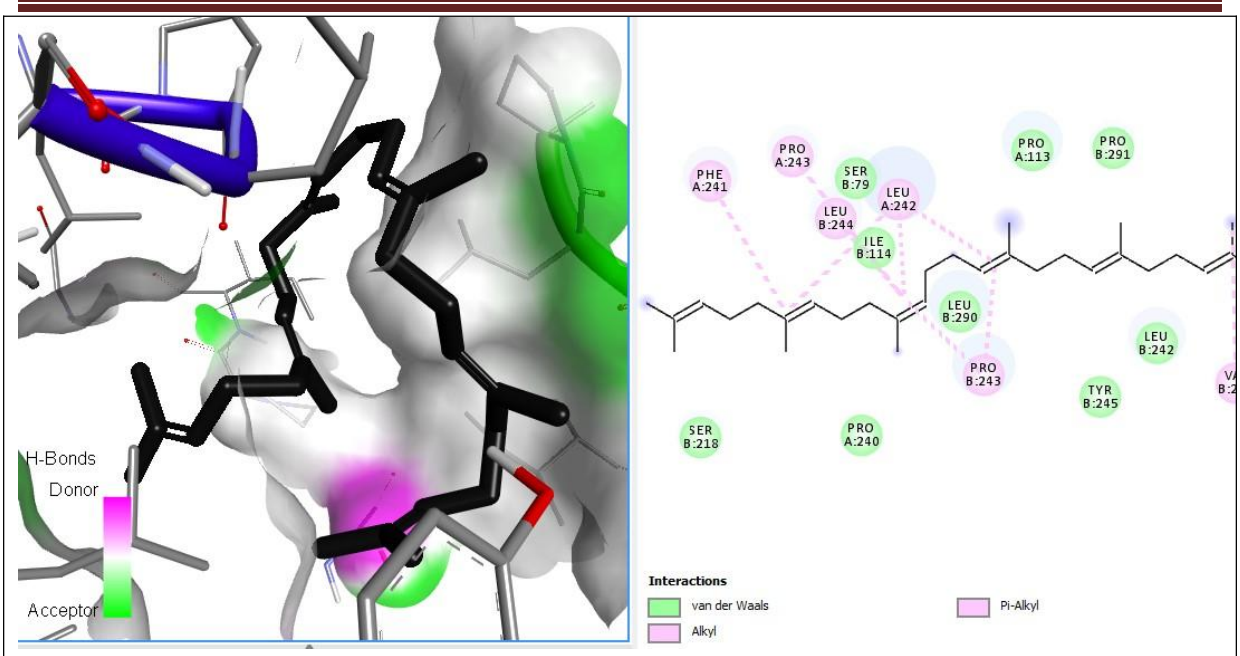


3D and 2D Interactions of Squalene and Falcipain 2 (-7.0 kcal/mol)

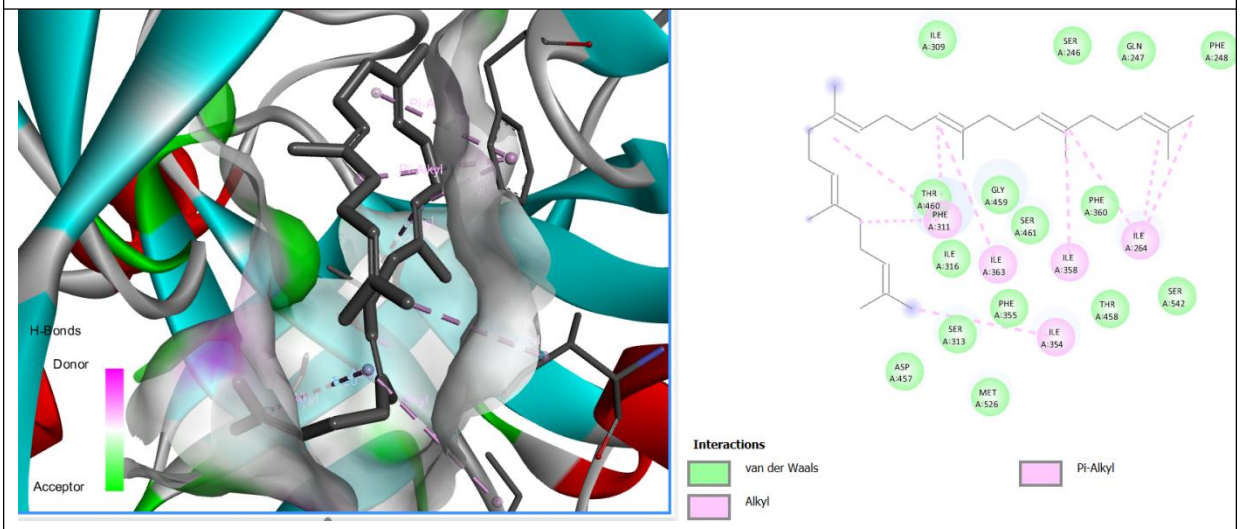


3D and 2D Interactions of Squalene and Falcipain 3 (-7.3 kcal/mol)

Figure 7a: Molecular Interactions of Squalene with some Receptors of *P. falciparum*

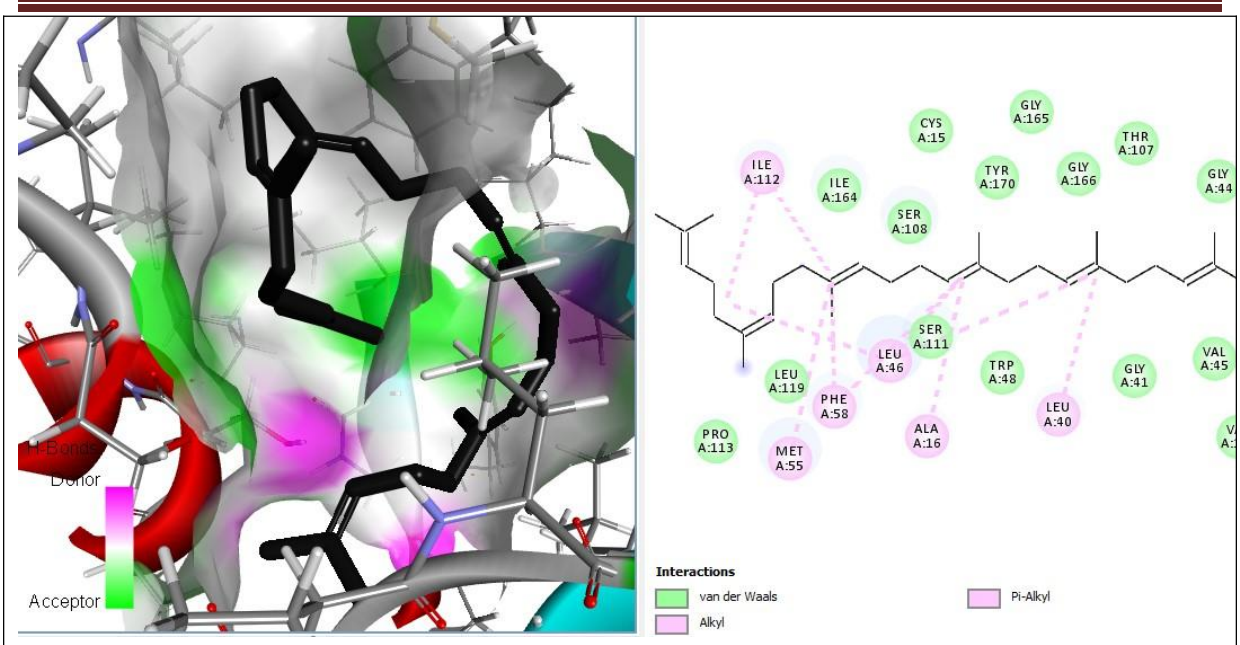


3D and 2D Interactions of Squalene and Plasmepsin IV (-7.1 kcal/mol)

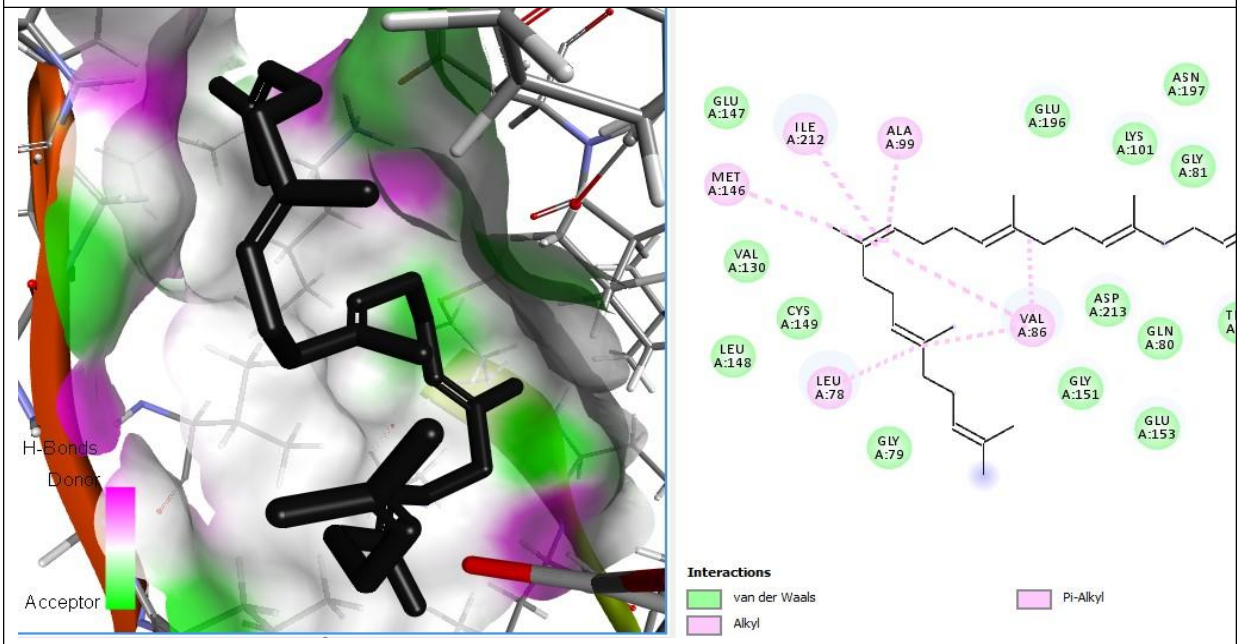


3D and 2D Interactions of Squalene and Plasmepsin X (-7.8 kcal/mol)

Figure 7b: Molecular Interactions of Squalene with some Receptors of *P. falciparum*

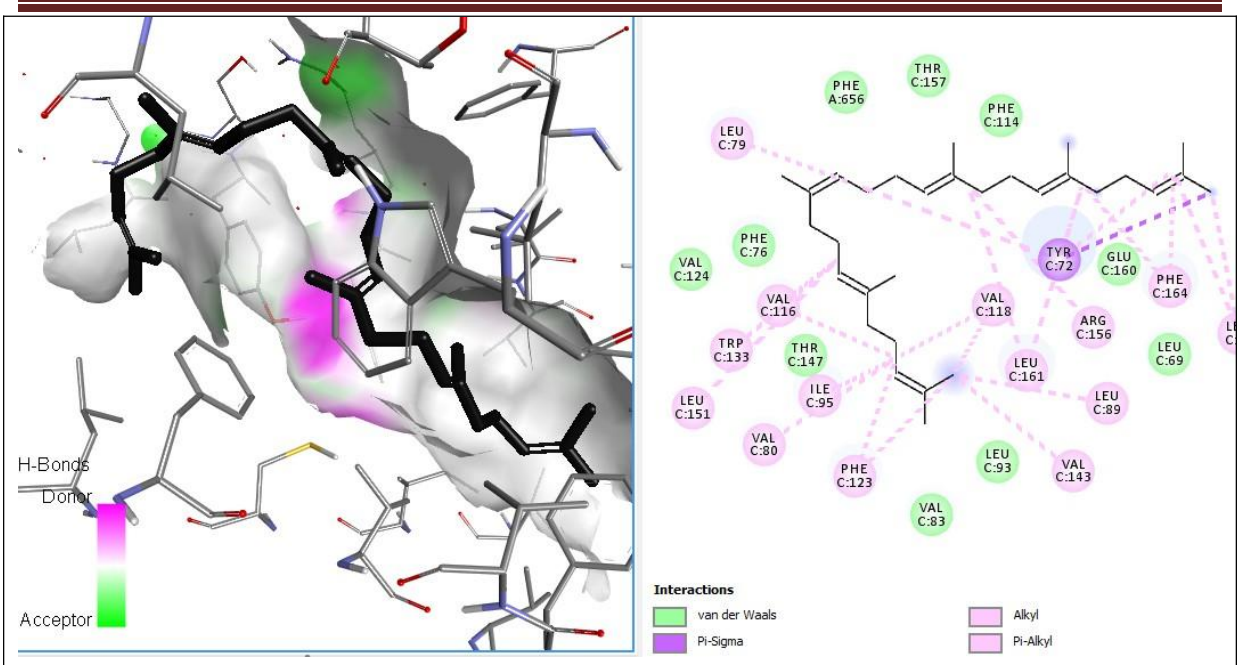


3D and 2D Interactions of Squalene and Plasmodium falciparum Dihydrofolate Reductase (-7.1 kcal/mol)



3D and 2D Interactions of Squalene and Plasmodium falciparum Calcium-Dependent Protein Kinase 1 (-7.9 kcal/mol)

Figure 7c: Molecular Interactions of Squalene with some Receptors of *P. falciparum*



3D and 2D Interactions of Squalene and Plasmodium falciparum Erythrocyte Membrane Protein 1 (-11.1 kcal/mol)

Figure 7d: Molecular Interactions of Squalene with some Receptors of *P. falciparum*

Falcipain 3 (-7.3 kcal/mol), Plasmepsin IV (-7.1 kcal/mol), Plasmepsin X (-7.8 kcal/mol), Plasmodium falciparum Dihydrofolate Reductase (-7.1 kcal/mol), Plasmodium falciparum Calcium-Dependent Protein Kinase 1 (-7.9 kcal/mol), and Plasmodium falciparum Erythrocyte Membrane Protein 1 (-11.1 kcal/mol). The interaction with Falcipain 2 involved several amino acid residues which include ASN 16, ASN 38, GLU 15, HIS 174, TRP 206, VAL 152, ARG 12, LYS 37, TRP 210, and HIS 19 using Van der Waals, Pi-sigma, Alkyl, and Pi-Alkyl bond. The interaction with Falcipain 3 involved LEU 47, TRP 215, ASP 44, ALA 166, PHE 167, CYS 48, and ASP 163 using Van der Waals, Pi-sigma, Alkyl, and Pi-Alkyl bond. The interaction with Plasmepsin IV involved PHE 241, PRO 243, LEU 244, ILE 114, LEU 242, VAL 292, and SER 218 using Van der Waals, Alkyl, and Pi-Alkyl bond. The interaction with Plasmepsin X involved THR 460, PHE 311, ILE 363, GLY 459, SER 461, ILE 264, and SER 542 using Van der Waals, Alkyl, and Pi-Alkyl bond. On the other hand, the interaction with Plasmodium falciparum Dihydrofolate Reductase involved ILE 112, LEU 119, MET 55, PHE 58, LEU 46, SER 111, ALA 16, ILE 164, SER 108, TRP 48, and LEU 40 using Van der Waals, Alkyl, and Pi-Alkyl bond. The interaction with Plasmodium falciparum Calcium-Dependent Protein Kinase 1 involved MET 146, ILE 212, ALA 99, VAL 86, LEU 78, and GLU 196 using Van der Waals, Alkyl, and Pi-Alkyl bond while the interaction with

Plasmodium falciparum Erythrocyte Membrane Protein 1 involved LEU 79, VAL 80, PHE 123, and PHE 114 using Van der Waals, Pi-sigma, Alkyl, and Pi-Alkyl bond.

The molecular docking results revealed six ligands that consistently exhibited the lowest binding affinity to thirteen receptors. These ligands, including phthalic acid 5-methylhex-2-yl butyl ester, 1,1'-bicyclohexyl 2-(2-methylpropyl) trans-, 9,12-octadecadienoic acid methyl ester, phytol, squalene, and Heneicosane 11-pentyl-, demonstrated binding affinities ranging between -7.0 and -11.1 kcal/mol. Notably, phthalic acid 5-methylhex-2-yl butyl ester, 1,1'-bicyclohexyl 2-(2-methylpropyl) trans-, and squalene exhibited favorable interactions with multiple targets, suggesting their potential to overcome drug resistance.

Specifically, phthalic acid, 5-methylhex-2-yl butyl ester demonstrated anti-plasmodial activity in this investigation by potentially impeding the growth or reproduction of *P. falciparum* through interactions with Plasmepsin X (-7.1 kcal/mol), the amino acids involved in the interactions were ILE 264, GLY 459, SER 461, PHE 311, ILE 358, SER 313, and ILE 354 using Van der Waals, Conventional hydrogen bond, Carbon-hydrogen bond, Pi-sigma, Alkyl and pi-Alkyl. By inhibiting Plasmepsin X, an aspartyl protease enzyme, Phthalic acid, 5-methylhex-2-yl butyl ester disrupts hemoglobin digestion, thereby limiting nutrient uptake by the parasite [20, 21]. Phthalic acid, 5-methylhex-2-yl butyl ester has also inhibited Plasmodium falciparum Dihydrofolate Reductase (-7.1 kcal/mol), by interacting with GLY 44, SER 108, LEU 40, LEU 46, MET 104, ILE 112, PHE 58, and ILE 164 using Van der Waals, Conventional hydrogen bond, Carbon-hydrogen bond, Alkyl and pi-Alkyl. Inhibition of Plasmodium falciparum DHFR impedes DNA and RNA synthesis, thereby preventing parasite replication [22, 23]. Additionally, Phthalic acid, 5-methylhex-2-yl butyl ester has also inhibited Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.1 kcal/mol), by interacting with TYR 72, PHE 164, PHE 114, LEU 161, and GLY 68 using Van der Waals, Carbon-hydrogen bond, Pi-pi stacked, Alkyl and pi-Alkyl. Inhibiting Plasmodium falciparum PfEMP1 disrupts the parasite's ability to adhere to host cells, thereby impeding its infectivity and ability to cause complications such as cerebral malaria [24]. Consequently, this multi-targeted approach offers advantages in combating *P. falciparum*, decreasing the likelihood of the parasite developing resistance to the compound [25]. Hence, the significant binding affinities noted between Phthalic acid, 5-methylhex-2-yl butyl ester, and the proteins further reinforce its potential as a promising anti-malarial agent.

1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer exhibits anti-malarial activity by disrupting the metabolic processes or cell structures of the parasite through the inhibition of Plasmepsin X (-7.3 kcal/mol), by interacting with ILE 264, PHE 360, GLN 247, GLY 459,

ILE 354, SER 246, and ILE 358 using Van der Waals, and Alkyl bond. By inhibiting Plasmepsin X, 1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer disrupts the parasite's ability to break down hemoglobin, which is essential for its survival and growth [26]. 1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer has also inhibited Plasmodium falciparum Dihydrofolate Reductase (-7.3 kcal/mol), by interacting with LEU 40, ALA 16, PHE 58, ILE 164, CYS 15, ASP 54, ILE 14, and MET 55 using Van der Waals, Alkyl, and Pi-Alkyl bond. Inhibiting Dihydrofolate Reductase prevents the parasite from breaking down hemoglobin, thereby hindering its ability to replicate and multiply within the host. 1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer has also inhibited Plasmodium falciparum multidrug resistant Protein 1 (-7.2 kcal/mol), by interacting with ILE 282, ILE 286, LEU 327, ILE 205, MET 330, PHE 202, LEU 333, and TYR 206 using Van der Waals, Alkyl, and Pi-Alkyl bond. Inhibiting PfMDR1 as a protein involved in resistance in *P. falciparum* can enhance the effectiveness of anti-malarial drugs and prevent the parasite from developing resistance to treatment [26]. Thus, by targeting these key proteins, 1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer interferes with the vital metabolic and cellular processes of the parasite. This multi-targeted approach is advantageous in combating malaria because it reduces the likelihood of the parasite developing resistance to the compound. The detected binding energies between 1,1'-Bicyclohexyl, 2-(2-methylpropyl)-, trans-isomer and these proteins support its potential as an effective anti-malarial agent. 9,12-Octadecadienoic acid, methyl ester (Linoleic acid) is an essential fatty acid known for its potential anti-malarial properties.

In this investigation, it has exhibited inhibition against Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.0 kcal/mol), by interacting with LEU 89, THR 147, VAL 80, VAL 116, ILE 95, VAL 83, LEU 93, PHE 123, VAL 143, VAL 118, TRP 133, LEU 79, VAL 124, LEU 151, TYR 72, PHE 114, ARG 156, PHE 76, LEU 161, and THR 157 using Van der Waals, Alkyl, and Pi-Alkyl bond. PfEMP1 is a protein crucial for the adherence of infected red blood cells to host cells, particularly in the development of severe malaria complications like cerebral malaria [27]. By inhibiting PfEMP1, 9,12-Octadecadienoic acid, methyl ester may prevent the parasite from effectively adhering to and infecting host cells, ultimately hindering its survival and progression. Inhibiting PfEMP1 is particularly significant in reducing the severity of malaria and preventing complications [28]. This mechanism supports the potential of 9,12-Octadecadienoic acid, methyl ester as a promising anti-malarial agent.

Phytol is a diterpene alcohol known for its reported anti-malarial activity. In this study, it has demonstrated inhibition against Plasmodium falciparum Erythrocyte Membrane Protein

1 (-8.3 kcal/mol), by interacting with VAL 83, VAL 80, LEU 89, THR 149, ILE 95, VAL 124, PHE 123, VAL 116, LEU 79, PHE 76, LEU 151, LEU 161, TYR 72, and ARG 156 using Van der Waals, Conventional hydrogen bond, Alkyl, and Pi-Alkyl bond. By inhibiting PfEMP1, Phytol disrupts the parasite's ability to adhere to host cells, thereby impeding its infectivity and reducing the risk of complications [29]. Inhibiting PfEMP1 is particularly significant in reducing the severity of malaria and preventing complications. This mechanism indicates the potential of Phytol as a promising anti-malarial agent.

Heneicosane, 11-pentyl- is a long-chain hydrocarbon identified for its potential as an anti-malarial agent in this study. The compound has exhibited inhibition against Plasmodium falciparum Erythrocyte Membrane Protein 1 (-8.1 kcal/mol), by interacting with PHE 164, TYR 72, LEU 161, PHE 656, THR 157, LEU 99, PHE 114, TRP 133, LEU 79, LEU 89, PHE 76, VAL 116, THR 147, VAL 83, VAL 80, PHE 123, and VAL 124 using Van der Waals, Pi-sigma, Alkyl, and Pi-Alkyl bond. This means that Heneicosane, 11-pentyl- may have prevented the parasite from effectively adhering to and infecting host cells, ultimately hindering its survival and progression [29].

Squalene, a natural compound found in various plants and animals, including humans, has shown inhibition against key proteins crucial for the survival and replication of Plasmodium falciparum in this study. It inhibits Falcipain 2 (-7.0 kcal/mol) by interacting with ASN 16, ASN 38, GLU 15, HIS 174, TRP 206, VAL 152, ARG 12, LYS 37, ALA 157, GLN 209, GLU 14, TRP 210, and HIS 19 using Van der Waals, Pi-sigma, Alkyl, and Pi-Alkyl bond. It also inhibits Falcipain 3 (-7.3 kcal/mol) by interacting with LEU 47, TRP 215, ASP 44, ALA 46, LEU 47, ALA 166, PHE 167, CYS 48, and ASP 163 using Van der Waals, Pi-sigma, Alkyl, and Pi-Alkyl bond. These cysteine proteases are essential for hemoglobin digestion in the parasite and by blocking these enzymes, squalene disrupts the breakdown of hemoglobin, vital for the parasite's growth [30]. Additionally, Squalene inhibits Plasmepsin IV (-7.1 kcal/mol) by interacting with PHE 241, PRO 243, LEU 244, ILE 114, LEU 242, PRO 243, PRO 113, TYR 245, LEU 242, VAL 292, and SER 218 using Van der Waals, Alkyl, and Pi-Alkyl bond. It also inhibits Plasmepsin X (-7.8 kcal/mol) by interacting with THR 460, PHE 311, ILE 363, GLY 459, SER 461, ILE 358, ILE 354, SER 313, ASP 457, MET 526, ILE 358, PHE 360, ILE 264, and SER 542 using Van der Waals, Alkyl, and Pi-Alkyl bond. These aspartic protease enzymes are involved in hemoglobin digestion and thus, inhibit them and further limit the parasite’s nutrient uptake [31].

Squalene also inhibits Plasmodium falciparum Dihydrofolate Reductase (-7.1 kcal/mol), by interacting with ILE 112, LEU 119, MET 55, PHE 58, LEU 46, SER 111, ALA

16, ILE 164, SER 108, TRP 48, and LEU 40 using Van der Waals, Alkyl, and Pi-Alkyl bond. PfDHFR is crucial for DNA and RNA synthesis and thus, this hinders the parasite's ability to replicate within the host. Furthermore, squalene inhibits Plasmodium falciparum Calcium-Dependent Protein Kinase 1 (-7.9 kcal/mol), by interacting with MET 146, ILE 212, ALA 99, VAL 86, LEU 78, TYR 83, ASP 213, GLY 151, LYS 101, and GLU 196 using Van der Waals, Alkyl, and Pi-Alkyl bond). By blocking CDPK1, squalene disrupts the parasite's normal cellular functions which regulates various cellular processes in the parasite, potentially leading to its death [32].

Significantly, squalene inhibits Plasmodium falciparum erythrocyte membrane Protein 1 (-11.1 kcal/mol), by interacting with LEU 79, VAL 116, TRP 133, LEU 151, THR 147, ILE 95, VAL 80, PHE 123, VAL 118, VAL 83, LEU 93, VAL 143, LEU 89, LEU 161, TYR 72, ARG 156, GLU 160, PHE 164, LEU 69, LEU 99, and PHE 114 using Van der Waals, Pi-sigma, Alkyl, and Pi-Alkyl bond). By inhibiting PfEMP1, squalene prevents the parasite from attaching to host cells, reducing its infectivity and the risk of complications such as cerebral malaria [33]. Thus, these results suggest that squalene's anti-malarial activity is multi-faceted targeting various crucial proteins involved in the parasite's survival, growth, and ability to infect host cells. This multi-targeted approach makes squalene a promising candidate for further development as an effective anti-malarial agent.

CONCLUSION

GC/MS analysis identified eighteen bioactive compounds, notably phthalic acid, 5-methylhex-2-yl butyl ester; 1,1'-bicyclohexyl, 2-(2-methylpropyl)-, trans-; 9,12-octadecadienoic acid methyl ester; phytol; squalene; and Heneicosane, 11-pentyl-, which recorded the lowest binding affinity (between -7.0 and -11.1 kcal/mol) among the eighteen compounds to important target receptors of *P. falciparum*. The predicted interactions between these compounds, notably squalene, and the receptors revealed multiple potential mechanisms of action, including cell membrane disruption, metabolic pathway inhibition, immune response modulation, and interference with nutrient acquisition. These findings highlight the potential of *J. tanjorensis* as a valuable candidate for developing effective anti-malarial agents, with squalene warranting further investigation. Further studies should also be conducted to isolate, and evaluate the anti-plasmodial activity and explore the molecular dynamic simulation of the bioactive compounds identified in *Jatropha tanjorensis*, with particular emphasis on squalene, better to understand their mechanisms of action and potential synergistic effects.

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