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Full-Length Research Article

In Silico Exploration of Antimalarial Potential of Ethanolic Leaf Extract of *Heliotropium indicum*

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Summary: *Heliotropium indicum,* commonly known as Indian heliotrope, is a member of the family *Boraginaceae,* and possessed array of ethnomedicinal usefulness. The objective of this study was to computationally investigate phytochemical constituents, pharmacokinetics and binding dynamics of ethanolic leaves extract of *H. indicum* with key molecular targets relevant to malarial. The method used are pharmacokinetics prediction, target prediction, and molecular docking. The results showed twenty-nine (29) identified constituents of which some are poorly soluble and have low gastrointestinal absorption (GIA) and others soluble with high GIA and could permeate the blood brain barrier (BBB). The molecular docking results showed that eicosane have the highest binding affinity for Enoyl- acyl-carrier protein (ACP) reductase (-7.363 kcal.mol^{-1}), followed by 3-oxoacyl-ACP reductase (-7.012 kcal.mol^{-1}), and beta-hydroxyacyl-ACP dehydratase (-6.295 kcal.mol^{-1}), while Nonadecane has binding affinity of -5.048 kcal.mol^{-1} for Heat shock protein 90; 2-(Pentyloxycarbonyl) benzoic acid has binding affinity of -5.375 kcal.mol^{-1} for cysteine protease falcipain-3, and 4-Methoxyanthranilic acid has binding affinity of -5.667 kcal.mol^{-1} for M17 leucyl aminopeptidase. Overall, this study showed that nonadecane, 1-chlorononadecane, eicosane, 2-(Pentyloxycarbonyl) benzoic acid, and 4-Methoxyanthranilic acid, are the five most active constituents of ethanolic leaves extract of *H. indicum*; targeting key proteins in *P. falciparum*. In conclusion, ethanolic leaves extract of *H. indicum* plant has potential antimalarial properties. However, further *in vivo* and *in vitro* studies are required to validate the claims of this study in order to established therapeutic efficacy of active compounds of ethanolic leaves extract of *H. indicum* that are directly responsible for its antimalarial properties.

Keywords*: Heliotropium indicum*, phytochemicals, pharmacokinetics, *Plasmodium falciparum*, Enoyl-ACP reductase, M17 leucyl aminopeptidase, molecular docking

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INTRODUCTION

Heliotropium indicum, is a member of the family *Boraginaceae,* consisting of about 250 species, which are distributed across warm temperate tropical, and subtropical zones of the world (Faleye *et al*., 2023). *H. indicum* is not widely known and consumed but it is of high nutritional quality and has antioxidant potential (Amusan *et al.*, 2016). Ethnomedicinal uses of *H. indicum* include treatment for malaria, ulcers, diarrhoea, fever, sore throat, whooping cough, wound, dermatitis and nervous disorders (Faleye *et al*., 2023). The pharmaceutical potentials of *H. indicum* are related to its chemical constituents, consequently responsible for their extensive bioactivities indicated in folk medicine (Koffuor *et al*., 2012; Boye *et al*., 2012). Our previous study has shown that *H. indicum* contained some phytochemicals (such as (+)-Isomenthol, and (3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol), that positioned it as potential treatment for prostate cancer (Faleye et al., 2023).

Malaria is the most significant parasitic disease of humans majorly caused by the apicomplexan parasites of the genus Plasmodium, with over a third of the world's population considered at risk, and an estimated 625,000 people die in year 2020 as a consequence of infection (World Health Organization, 2021). The widespread appearance of drug-resistant malaria parasites, even to newly developed second and third generation therapeutics such artemisinin and its derivatives, makes the development of unique antimalarial drug more urgent (Fatoki *et al*., 2022).

Computational approaches have been used to investigate the phytochemicals from medicinal plants in order to unraveled the pharmacokinetics and molecular targets which are modulated in ameliorating specific human diseases (Fatoki *et al*., 2023; Faleye *et al*., 2023). The objective of this study was to computationally investigate the ethanolic leaves extract of *H. indicum* for its phytochemicals, and assessing its pharmacokinetics and binding dynamics with key molecular targets relevant to malarial.

MATERIALS AND METHODS

Plant sample: *H. indicum* plant sample was harvested from the Federal University of Technology Akure, Ondo State, Nigeria. The raw sample was identified and authenticated at

the Herbarium center, Ekiti State University, Ado-Ekiti Nigeria with Herbarium No: UHAE 2021379. Fresh, green and undamaged leaves harvested were air-dried until constant weight was obtained. After proper drying, the leaves were blended into fine powder.

Chemicals and reagents: All chemicals and reagents used were of analytical grade from Sigma Aldrich (USA).

Determination of *H. indicum* Phytochemical by Gas Chromatography – Mass Spectrometry (GC-MS): The sample treatment and identification of phytochemicals in the ethanolic leaves extract of *H. indicum* was carried out as previously reported by Faleye *et al* (2023).

Ligand preparation: The chemical structures of phytochemicals (ligands) found in ethanolic leaves extract of *H. indicum* were obtained from NCBI PubChem Compound database (https://pubchem.ncbi.nlm.nih.gov/) in SMILES and 3D SDF format.

***In Silico* ADME Prediction:** The *in silico* absorption, distribution, metabolism, and excretion (ADME) screening of the compounds were carried out on SwissADME server, available at www.swissadme.ch (Daina *et al.,* 2017).

***In Silico* Molecular Target Prediction**: The simplified molecular input line entry system (SMILES) of each of the ligands were used for the target prediction analysis on the SEA Search Server (http://www.sea.bkslab.org/) (Keiser *et al*., 2007), and molecular targets for *Plasmodium falciparum* was selected for further investigation.

Molecular docking studies: The SMILES of the ligands were subjected to 3D structure optimization using ACDLab/Chemsketch software, and saved in .mol format. Subsequently, PyMol software was utilized for the conversion of ligand files from .mol to .pdb format. The 3D structures of identified molecular targets of *Plasmodium falciparum*, were obtained as AlphFold pdb format from UniProt database. Prior to docking, the ligands and target proteins structures in pdb format were formatted to pdbqt by using AutoDock Tools (ADT) v1.5.6 (Morris *et al*., 2009). Ligand-protein molecular docking was carried out using AutoDock Vina v1.2.3 (Trott and Olson, 2010; Eberhardt *et al*., 2021), as previously reported (Fatoki *et al*., 2023). Afterward, binding affinity was obtained and the outputs of ligand-proteins interaction poses were visualized on PoseView webserver available at https://proteins.plus/ (Stierand *et al*., 2006)

RESULTS

The twenty-nine (29) identified constituents of *Heliotropium indicum* ethanolic leaves extract from GC-MS are shown in Table 1 which include Thunbergol, 1,4-Eicosadiene, (3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol, Nonadecane, Phytol, (+)-Isomenthol, 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene, 1-chloro-Nonadecane, Eicosane and Octadecane. The results of pharmacokinetics are shown in Table 2, which suggest that many of the constituents are poorly soluble and have low gastrointestinal absorption (GIA), these include ergost-22-en-3-ol, neophytadiene and 1,4-eicosadiene. Furthermore, some are soluble with high GIA, which could pervade blood-brain barrier (BBB) and some were not substrate of P-glycoprotein (P-gp).

The results of target prediction showed that eight (8) compounds (9-Octadecyne, Nonadecane, 1-chlorononadecane, Eicosane, Octadecane, (Z)-3-Heptadecen-5-yne, 2-(Pentyloxycarbonyl) benzoic acid, and 4-Methoxyanthranilic acid) targeted *Plasmodium falciparum* proteins which include Enoyl-ACP reductase, 3-oxoacyl-acyl-carrier protein reductase, and Beta-hydroxyacyl-ACP dehydratase (Table 3). The results were ranked based on p-value and maximum tanimoto coefficient (MTC), which give similarity between compounds from reference target and query target, where highest similarity is 1.

The 8 compounds of *Heliotropium indicum* ethanolic leaves extract with potential antimalarial effect, were subjected to molecular docking analyses and the results presented in Table 4 showed that eicosane have the highest binding affinity for enoyl-ACP reductase (-7.363 kcal.mol^{-1}), followed by 3-oxoacyl-ACP reductase (-7.012 kcal.mol^{-1}), and beta-hydroxyacyl-ACP dehydratase (-6.295 kcal.mol^{-1}), while nonadecane has binding affinity of -5.048 kcal.mol^{-1} for Heat shock protein 90; 2-(Pentyloxycarbonyl) benzoic acid has binding affinity of -5.375 kcal.mol^{-1} for cysteine protease falcipain-3, and 4-methoxyanthranilic acid has binding affinity of -5.667 kcal.mol^{-1} for M17 leucyl aminopeptidase. The binding pose of the complex with high binding affinity are presented in Figure 1, which showed the amino acid residues involved in the binding interaction. The result showed that eicosane, nonadecane and 1-chlorononadecane, did not have specific interaction with the amino acid residues of the protein targets.

DISCUSSION

Gas chromatography-mass spectrometry (GC–MS) is a novel technique to identify the secondary metabolites (such as alkaloids, terpenes, flavonoids, various glycosides, and short or long hydrocarbons, e.t.c.) from the plants, and it is reliable for identification of the compound present in complex biochemical product (Muthulakshmi *et al*., 2012; Ahsan *et al*., 2017). Some of phytochemicals identified with GC-MS in this study include 6,10,14-trimethyl 2-Pentadecanone, 9-Octadecyne, Nonadecane, 1-chlorononadecane, Eicosane, Octadecane, (Z)-3-Heptadecen-5-yne, 2-(Pentyloxycarbonyl)benzoic acid, and 4-Methoxyanthranilic acid.

The pharmacokinetics results indicated that most of the phytoconstituents of *H. indicum* have high solubility and gastrointestinal absorption (GIA), some could permeate the BBB, serve as P-gp substrates and inhibit the action of some cytochromes (CYPs). The interplay of GIA, BBB penetration, P-glycoprotein modulation, and cytochrome inhibition collectively influences the pharmacokinetics and pharmacodynamics of phytochemicals or bioactive compounds. Enhancement or inhibition of CYPs activities can change drug metabolism profile and result in either increase in the bioavailability or decrease in the efficacy of the drug (Martin *et al*., 2013). The toxicity of the drug systems decreases as the Log P value decreases, and systems with a Log P value of around 2.2 are more suitable for oral bioavailability (Arnott and Planey, 2012).

Table 1:
GC-MS analysis of phytochemnical constituents of ethanolic leaves extract of *Heliotropium indicum*

SN	Identified Constituents	Retention Time	Area	Reference	Quality (%)
1	Neophytadiene	10.509	10.52	138502	99
2	2,6,6-trimethyl-bicyclo[3.1.1]heptane,	10.509	10.52	17379	91
3	1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-ylmethylphenylsulfoxide	10.553	1.54	188513	56
4	6,10,14-trimethyl 2-Pentadecanone	10.553	1.54	128827	49
5	Thunbergol	10.553	1.54	150221	46
6	(Z,E)-9,12-Tetradecadien-1-ol	10.613	2.94	74499	68
7	6-Octen-1-ol, 3,7-dimethyl-, acetate	10.613	2.94	63306	55
8	9-Octadecyne	10.694	4.59	111836	83
9	1,4-Eicosadiene	10.694	4.59	138502	66
10	(3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol	10.894	1.26	239012	55
11	3,7,11-trimethyl-, (Z,E)-2,6,10-Dodecatrien-1-ol	10.894	1.26	85762	55
12	Nonadecane	10.894	1.26	128834	53
13	Phytol	11.701	36.37	155849	94
14	(+)-Isomenthol	11.701	36.37	29169	60
15	Dodecahydropyrido[1,2-b]isoquinolin-6-one	11.849	4.45	71593	42
16	Fumaric acid, 2,4,6-trichlorophenyl tridecyl ester	11.849	4.45	262552	30
17	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	11.849	4.45	141906	25
18	1-chloro-Nonadecane	11.931	7.95	161769	93
19	Eicosane	11.931, 14.457	7.95, 3.12	142239	68, 92
20	Octadecane	11.931, 14.457	7.95, 3.12	115546	64, 92
21	n-Propyl 9,12,15-octadecatrienoate	11.968	6.86	179097	97
22	3-ethenyl-Cyclooctene	11.968	6.86	16103	50
23	(Z)-3-Heptadecen-5-yne	11.968	6.86	96839	46
24	28-Nor-17.beta.(H)-hopane	12.205	8.59	237949	89
25	(1R,2S,8R,8Ar)-8-acetoxy-1-(2-hydroxyethyl)-1,2,5,5-tetramethyl-trans-decalin	12.205	8.59	155648	83
26	28-Nor-17.alpha.(H)-hopane	12.205	8.59	237950	64
27	Bis(2-ethylhexyl) phthalate	14.745	11.32	233373	87
28	2-(Pentyloxycarbonyl)benzoic acid	14.745	11.32	98123	64

Table 2:
Predicted ADME of phytochemicals of ethanolic leaves extract of *Heliotropium indicum* from GC-MS analysis

SN	Identified Constituents	Pubchem CID	Predicted ADME Parameters									
			Physicochemical properties			Lipophilicity	Water solubility		Pharmacokinetics			Drug-likeness
			MW	MR	TPSA (Å²)	Log P	ESOL Log S	ESOL Class	GIA	BBB	P-gp	BS
1	Neophytadiene	10446	278.52	97.31	0.00	7.07	-6.77	Poor	Low	No	Yes	0.55
2	2,6,6-trimethyl-bicyclo[3.1.1]heptane	91015300	138.25	45.7	0.00	3.38	-3.15	Soluble	Low	Yes	No	0.55
3	1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-ylmethylphenylsulfoxide	565587	330.53	100.93	36.28	5.23	-5.89	Moderate	High	No	No	0.55
4	6,10,14-trimethyl 2-Pentadecanone	10408	268.48	88.84	17.07	5.66	-5.09	Moderate	High	No	Yes	0.55
5	Thunbergol	5363523	290.48	95.92	20.23	4.75	-4.77	Moderate	High	No	No	0.55
6	(Z,E)-9,12-Tetradecadien-1-ol	5365760	210.36	69.63	20.23	4.16	-3.45	Soluble	High	Yes	No	0.55
7	6-Octen-1-ol, 3,7-dimethyl-, acetate	9017	198.3	60.61	26.30	3.46	-3.43	Soluble	High	Yes	No	0.55
8	9-Octadecyne	141998	250.46	86.8	0.00	6.79	-6.13	Poor	Low	No	No	0.55
9	1,4-Eicosadiene	5365774	278.52	97.31	0.00	7.34	-6.72	Poor	Low	No	No	0.55
10	(3.beta.,5.alpha.,22E,24R)-Ergost-22-en-3-ol	21159880	400.68	128.42	20.23	6.83	-7.63	Poor	Low	No	No	0.55
11	3,7,11-trimethyl-, (Z,E)-2,6,10-Dodecatrien-1-ol	445070	222.37	73.96	20.23	4.32	-4.17	Moderately	High	Yes	No	0.55
12	Nonadecane	12401	268.52	93.45	0.00	7.54	-6.69	Poor	Low	No	No	0.55
13	Phytol	5280435	296.53	98.94	20.23	6.22	-5.98	Moderate	Low	No	Yes	0.55
14	(+)-Isomenthol	45056	156.27	49.23	20.23	2.58	-2.88	Soluble	High	Yes	No	0.55
15	Dodecahydropyrido[1,2-b]isoquinolin-6-one	610048	207.31	65.27	20.31	2.48	-2.86	Soluble	High	Yes	No	0.55
16	Fumaric acid, 2,4,6-trichlorophenyl tridecyl ester	91694891	477.85	125.89	52.60	7.64	-7.99	Poor	Low	No	No	0.55
17	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	610255	282.29	78.02	52.60	2.86	-2.41	Soluble	High	Yes	No	0.55
18	1-chloro-Nonadecane	545634	302.97	98.24	0.00	7.68	-7.31	Poor	Low	No	No	0.55
19	Eicosane	8222	282.55	98.25	0.00	7.90	-7.05	Poor	Low	No	No	0.55
20	Octadecane	11635	254.49	88.64	0.00	7.18	-6.33	Poor	Low	No	No	0.55
21	n-Propyl 9,12,15-octadecatrienoate	12115559	320.51	102.92	26.3	6.26	-5.29	Moderate	Low	No	No	0.55
22	3-ethenyl-Cyclooctene	5365641	136.23	47.12	0.00	3.26	-3.23	Soluble	Low	Yes	No	0.55
23	(Z)-3-Heptadecen-5-yne	5367448	234.42	81.52	0.00	6.23	-5.55	Moderate	Low	No	No	0.55
24	28-Nor-17.beta.(H)-hopane	600852	384.68	125.36	0.00	7.86	-8.89	Poorl	Low	No	No	0.55
25	(1R,2S,8R,8Ar)-8-acetoxy-1-(2-hydroxyethyl)-1,2,5,5-tetramethyl-trans-decalin	6420608	296.44	86.34	46.53	3.61	-4.15	Moderate	High	Yes	No	0.55
26	28-Nor-17.alpha.(H)-hopane	18625	398.71	129.65	0.00	8.28	-9.31	Poor	Low	No	No	0.55
27	Bis(2-ethylhexyl) phthalate	8343	390.56	116.3	52.60	6.17	-6.06	Poor	High	No	Yes	0.55
28	2-(Pentyloxycarbonyl)benzoic acid	90531	236.26	63.91	63.60	2.73	-3.31	Soluble	High	Yes	No	0.85
29	4-Methoxyanthranilic acid	351010	167.16	44.3	72.55	0.49	-1.33	Very Sol	High	No	No	0.85

Legend: *Molecular weight (MW), Molar Refractivity (MR), Total polar surface area (TPSA). Consensus Log P, ESOL Log S, ESOL Class, Gastrointestinal absorption (GIA), Blood-brain barrier (BBB), P-glycoprotein (P-gp) substrate, Bioavailability Score (BS).*

Table 3:
P. falciparum molecular targets for the active constituents of ethanolic leaves extract of *Heliotropium indicum*

SN	Compound	Target name	Target gene code	Target description	p-value	MTC
8	9-Octadecyne	Q9BJJ9_PLAFA	FabI	Enoyl-ACP reductase	7.421e-35	0.57
		Q965D6_PLAFA	fabG	3-oxoacyl-ACP reductase	2.259e-17	0.57
		Q965D7_PLAFA	fabZ	Beta-hydroxyacyl-ACP dehydratase	1.643e-14	0.57
12	Nonadecane	Q9BJJ9_PLAFA	FabI	Enoyl-ACP reductase	9.993e-23	0.36
		Q965D6_PLAFA	fabG	3-oxoacyl-ACP reductase	1.859e-11	0.36
		Q965D7_PLAFA	fabZ	Beta-hydroxyacyl-ACP dehydratase	1.314e-09	0.36
		Q8IL32_PLAF7		Heat shock protein 90, putative	1.044e-06	0.53
18	1-chlorononadecane	Q9BJJ9_PLAFA	FabI	Enoyl-ACP reductase	1.126e-18	0.30
		Q965D6_PLAFA	fabG	3-oxoacyl-ACP reductase	1.76e-09	0.30
19	Eicosane	Q9BJJ9_PLAFA	FabI	Enoyl-ACP reductase	9.993e-23	0.36
		Q965D6_PLAFA	fabG	3-oxoacyl-ACP reductase	1.859e-11	0.36
		Q965D7_PLAFA	fabZ	Beta-hydroxyacyl-ACP dehydratase	1.314e-09	0.36
20	Octadecane	Q9BJJ9_PLAFA	FabI	Enoyl-ACP reductase	9.993e-23	0.36
		Q965D6_PLAFA	fabG	3-oxoacyl-ACP reductase	1.859e-11	0.36
		Q965D7_PLAFA	fabZ	Beta-hydroxyacyl-ACP dehydratase	1.314e-09	0.36
		Q8IL32_PLAF7		Heat shock protein 90, putative	1.044e-06	0.53
23	(Z)-3-Heptadecen-5-yne	Q9BJJ9_PLAFA	FabI	Enoyl-ACP reductase	2.727e-25	0.41
		Q965D7_PLAFA	fabZ	Beta-hydroxyacyl-ACP dehydratase	1.209e-10	0.41
28	2-(Pentyloxycarbonyl)benzoic acid	Q9NBA7_PLAFA		Cysteine protease falcipain-3	1.415e-10	0.28
		Q8II92_PLAF7	-	Deoxyuridine 5'-triphosphate nucleotidohydrolase	2.798e-06	0.34
29	4-Methoxyanthranilic acid	CYSP_PLAFA		Trophozoite cysteine proteinase	2.854e-64	0.35
		P90584_PLAFA	Pfmrk	MO15-related protein kinase Pfmrk	9.802e-09	0.34
		AMPL_PLAF7	-	M17 leucyl aminopeptidase	2.98e-07	0.35

MTC: maximum tanimoto coefficient.

Table 4:
Molecular docking results

SN	TARGETS	Docking parameters	Binding Affinity ΔG (kcal.mol^{-1})							
			C8	C12	C18	C19	C20	C23	C28	C29
1	Enoyl-ACP reductase (UniProt: Q9BJJ9)	Spacing: 0.700 Npts: 90 × 126 × 126 Center:0.746 × -14.020 × 0.866	-4.527	-5.843	-5.656	-7.363	-4.060	-3.688		
2	3-oxoacyl-ACP reductase (UniProt: Q965D6)	Spacing: 0.400 Npts: 126 × 126 × 126 Center: -3.494 × 6.559 × -3.310	-4.446	-6.099	-6.407	-7.012	-4.206			
3	Beta-hydroxyacyl-ACP dehydratase (UniProt: Q965D7)	Spacing: 0.450 Npts: 126 × 126 × 126 Center: 0.730 × -5.419 × 2.534	-4.206	-5.192		-6.295	-2.390	-4.487		
4	Heat shock protein 90, putative (UniProt: Q8IL32)	Spacing: 1.000 Npts: 126 × 126 × 126 Center: -5.284 × 3.381 × -0.794		-5.048			-3.970			
5	Cysteine protease falcipain-3 (UniProt: Q9NBA7)	Spacing: 0.600 Npts: 126 × 100 × 126 Center: -8.981 × -6.367 × 10.687							-5.375	
6	Deoxyuridine 5'-triphosphate nucleotidohydrolase (UniProt: Q8II92)	Spacing: 0.475 Npts: 126 × 96 × 110 Center: 3.919 × -1.334 × -3.364							-4.850	
7	Trophozoite cysteine proteinase (UniProt: P25805)	Spacing: 0.675 Npts: 126 × 126 × 90 Center: 9.754 × 2.555 × -0.533								-4.974
8	MO15-related protein kinase Pfmrk (UniProt: P90584)	Spacing: 0.525 Npts: 126 × 126 × 126 Center: -0.992 × 3.534 × -0.104								-5.494
9	M17 leucyl aminopeptidase (UniProt: Q8IL11)	Spacing: 0.625 Npts: 126 × 126 × 126 Center: -6.597 × 4.560 × -8.097								-5.667

***C8**: 9-Octadecyne. **C12**: Nonadecane. **C18**: 1-chlorononadecane. **C19**: Eicosane. **C20**: Octadecane. **C23**: (Z)-3-Heptadecen-5-yne. **C28**: 2-(Pentyloxycarbonyl) benzoic acid. **C29**: 4-Methoxyanthranilic acid.*

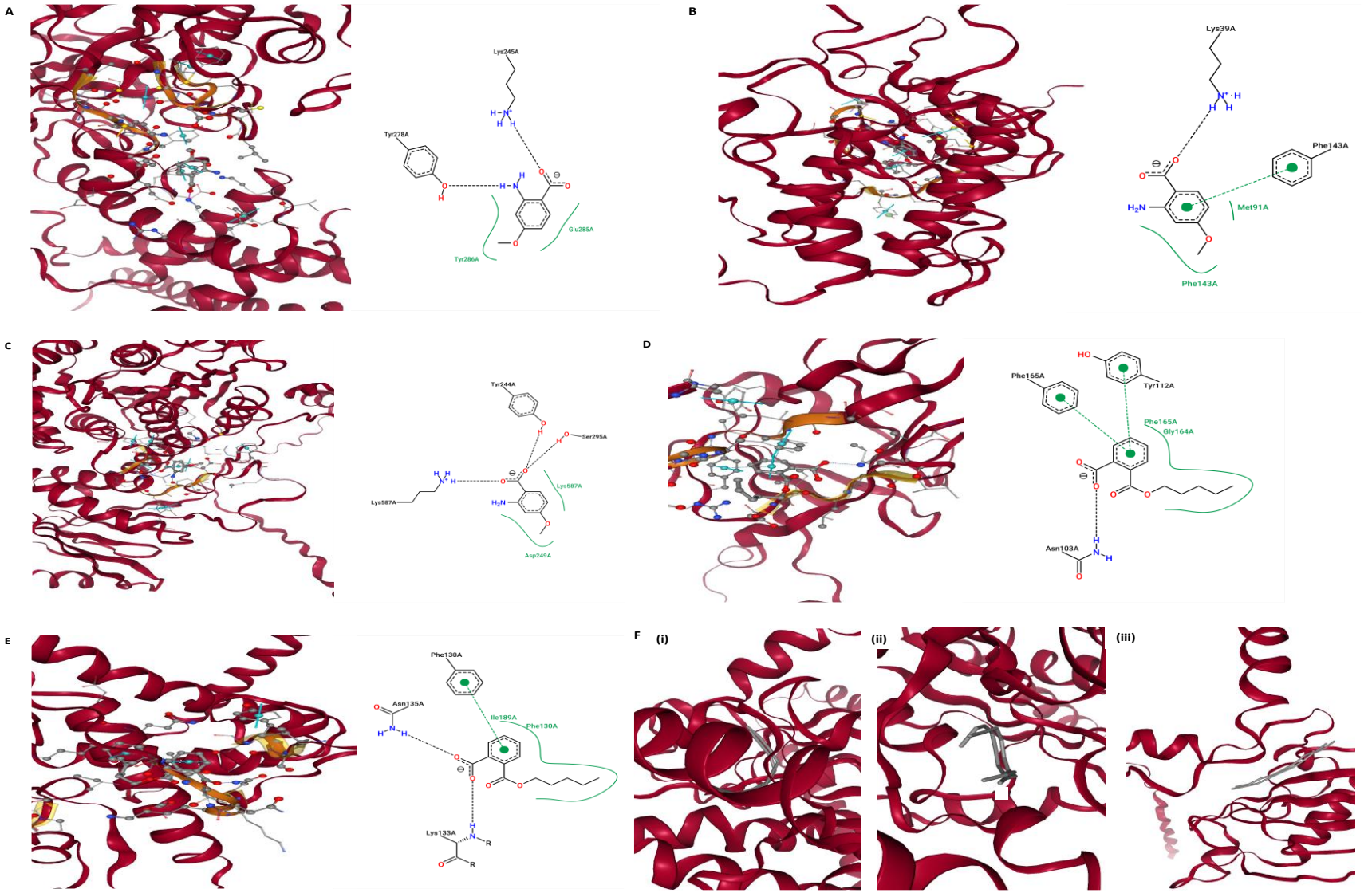

Figure 1: Selected molecular docking interactions, (**A**) C29 and P25805. (**B**) C29 and P90584. (**C**) C29 and Q8IL11. (**D**) C28 and Q8II92. **(E)** C28 and Q9NBA7. **(F)** (i) C19 and Q9BJJ9 (ii) C12 and Q9BJJ9. (iii) C12 and Q965D7. **Legend**: green straight line (hydrophobic contact), green dotted line (pi-pi contacts), and black dotted line (hydrogen bond).

In this study, the results showed that some phytochemicals present in the ethanolic leaves extract of *H. indicum* could modulates nine (9) *P. falciparum* proteins which can be grouped into three sets (i) Enoyl-ACP reductase (UniProt: Q9BJJ9), 3-oxoacyl-ACP reductase, (UniProt: Q965D6), and Beta hydroxyacyl-ACP dehydratase (UniProt: Q965D7), which are essential for the fatty acid synthesis in the parasite (Sharma *et al*., 2003; Nicola *et al*., 2007; Morde *et al*., 2009; Saeed *et al*., 2023). (ii) Cysteine protease falcipain-3 (UniProt: Q9NBA7), Trophozoite cysteine proteinase (UniProt: P25805), M17 leucyl aminopeptidase (UniProt: Q8IL11), which have been recognized as potential targets for antimalarial drug development due to their essential roles in the biology of these parasites (Gardiner *et al*., 2006; Pandey *et al*., 2006; Lê *et al*., 2022). (iii) Heat shock protein 90, putative (UniProt: Q8IL32), (iv) Deoxyuridine 5'-triphosphate nucleotidohydrolase (UniProt: Q8II92), and MO15-related protein kinase Pfmrk (UniProt: P90584).

Several studies have reported that Eicosane obtained from microbial sources, possessed antifungal effect (Nandhini, 2015; Ahsan *et al*., 2017). Thus, the mechanism by which eicosane exhibited antifungal effect could be similar to its anti-plasmodial effect in malarial disease. It has been reported that eicosane obtained from the crude extract of *Marantodes pumilum* possessed very strong anti-inflammatory, analgesic, and antipyretic effects (Okechukwu, 2020), as well as wound-healing properties possibly due to its good antioxidant properties (Balachandran *et al*., 2023). Therefore, the observed antimalarial activity of ethanolic leaves extract of *H. indicum* in this study may be partly due to the presence of eicosane in the extract, which modulates the activity of Enoyl-ACP reductase, 3-oxoacyl-ACP reductase and Beta-hydroxyacyl-ACP dehydratase. Eicosane is a long-chain hydrocarbons that has been found in plants such as *Taraxacum officinale*, *Hypericum hircinum* and *Acacia nilotica* (Chuah *et al*., 2018).

Nonadecane obtained from the plants and microbial sources have been reported for antibacterial effect (Tyagi and Agarwal, 2017; Kumari *et al*., 2019). Thus, the mechanism by which nonadecane exhibited antibacterial effect could be similar to its anti-plasmodial effect in malarial disease. Hence, the presence of nonadecane may contribute to the antimalarial properties of ethanolic leaves extract of this plant, which modulate the activity of Beta-hydroxyacyl-ACP dehydratase, Enoyl-ACP reductase, 3-oxoacyl-ACP reductase and Heat shock protein 90.

Anthranilic acid, which is derived from chorismic acid, is a nonessential amino acid, while chorismic acid, which is derived from shikimic acid, is also a precursor of phenylalanine and tyrosine, which are essential amino acids (Funayama and Cordell, 2015). 4-methoxyanthranilic acid is an organic compound that often required in large quantity for natural product synthesis, such as the production of anthracyclines and alkaloids. The main groups of alkaloids derived from anthranilic acid are classified as quinoline, acridone, and quinazoline alkaloids (Funayama and Cordell, 2015). Hence, the presence of 4-methoxyanthranilic may contribute to the antimalarial properties of ethanolic leaves extract of this plant, by modulating the activity of Trophozoite cysteine proteinase, MO15-related protein kinase Pfmrk, and M17 leucyl aminopeptidase. Plasmodium M17 leucyl aminopeptidase is one of the neutral aminopeptidases, that is essential for parasite viability, and function in the blood stage of the infection and responsible for regulating the intracellular amino acid pool within malaria cells, most likely by releasing amino acids from host-derived haemoglobin (McGowan *et al*., 2010; Mathew *et al*., 2021). The amino acid residues Met392, Met396, Phe398, Thr486, Gly489, Leu492, and Phe583 have been reported in the binding interaction of plasmodium M17 leucyl aminopeptidase (McGowan *et al*., 2010), while Arg202-Glu238 formed salt bridges and Phe231, Trp457, Trp461 involved in hydrophobic interactions in plasmodium cysteine protease falcipain-3 (Pant *et al*., 2018).

In conclusion, this work showed that nonadecane, 1-chlorononadecane, eicosane, 2-(Pentyloxycarbonyl) benzoic acid, and 4-Methoxyanthranilic acid, are the five most active constituents of ethanolic leaves extract of *H. indicum*; that relate to targeting key proteins in *P. falciparum*, which have implication in malarial. Thus, ethanolic leaves extract of *H. indicum* plant has antimalarial properties. However, further *in vivo* and *in vitro* studies are required to validate the claims of this study.

Conflict of Interest

All authors declare that there is no conflict of interest.

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