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In silico antiplasmodial effects of phytocompounds derived from *Andrographis paniculata* on validated drug targets of different stages of *Plasmodium falciparum*

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Author contributions

Funmilayo I. D. Afolayan conceived the idea and designed the study. Experiments and in silico analyses were done by Funmilayo I. D. Afolayan and Sayo Ebenezer Oladokun. Sayo Ebenezer Oladokun entered the data. Sayo Ebenezer Oladokun wrote the 1st draft which was critically reviewed by Funmilayo I. D. Afolayan.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

A. paniculata, Andrographis paniculata; PDB, Protein Data Bank; ADMET, Absorption, Distribution, Metabolism, Excretion, and Toxicity; 3D,three-dimensional structures; CASTp, Computed Atlas for Surface Topography of Proteins; MD, Mean Difference RMSD, Root Mean Square Deviation; RMSF, Root Mean Square Fluctuation; HGXPRT, hypoxanthine-guanine-xanthine phosphoribosyltransferase.

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Abstract

Background: *Andrographis paniculata* has been widely reported as an herbal plant for malaria treatment. The increasing rate of resistance to recommended antimalarial drugs has justified the need for a continuous search for new and more potent drugs that target all stages of the *Plasmodium falciparum* life cycle from natural plant sources. This study aimed to determine the antiplasmodial effect of phytocompounds derived from *A. paniculata* on the stages of *plasmodium falciparum*. **Methods**: Phytocompounds from *A. paniculata* were identified by Gas Chromatography-Mass Spectrophotometry (GCMS) analysis. The phytocompounds were screened for their druggability using Lipinski's rule of five and subjected to Absorption, Distribution, Metabolism, Excretion, Toxicity (ADMET) and druglikeness analysis. The phytocompounds were docked against some validated drug targets at different stages of *Plasmodium falciparum* (hepatic, asexual, sexual, and vector targets) using PyRx software to analyze the inhibitory potential and protein-ligand interaction. Thereafter, the stability and flexibility of the best complexes were assessed through molecular dynamics simulations at 50ns using WebGRO. **Result**: The 7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl exhibited a higher binding affinity and better stability throughout the simulation period with *P. falciparum* dihydrofolate reductase-thymidylate synthase and *Plasmodium falciparum* M1 alanyl aminopeptidase for asexual blood stage and gametocyte stage of *Plasmodium falciparum,* respectively than the existing drugs. Meanwhile, N-Ethyl-3-methoxy-4-methylphenethylamine was also found to have a higher binding affinity and more stability throughout the simulation period with *P. falciparum* purine nucleoside phosphorylase and *Plasmodium falciparum* gametocyte surface protein for Hepatic schizonts stage of *Plasmodium falciparum* and gametocyte transmission blocking stage, respectively, than the existing drugs. **Conclusion**: The 7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl and N-Ethyl-3-methoxy-4 methylphenethylamine from *A. paniculata* are predicted as an antimalarial drug candidate. Thus, it is recommended that *in vitro* and *in vivo* bioassays be conducted on these hit compounds to validate these predictions.

Keywords: *Plasmodium falciparum*; drug targets; *Andrographis paniculata*; molecular docking; molecular dynamics

Background

Malaria, a lethal disease caused by a microscopic parasite, is transmitted to humans through mosquito bites [1]. Malaria is caused by five species of mosquito-transmitted Plasmodium parasites: *P. falciparum, P. knowlesi, P. ovale*, *P. malariae*, and *P. vivax*. Among these, *P. falciparum* is responsible for the highest number of deaths, while infections caused by *P. vivax* and *P. knowlesi* can also result in severe illness [2]. Notably, infections with *P. vivax* and *P. ovale* may involve dormant liver-stage forms (hypnozoites) that can become active months or even years after the initial infection, leading to relapse and recurring disease [3]. According to the World Health Organization's World Malaria Report (2020), there is an increased risk of severe malaria, which can be fatal, for children under five years old and pregnant women. Furthermore, the report highlights that approximately 50% of the world's population lives in regions that are susceptible to malaria, encompassing 87 countries and territories.

Despite progress in recent years with the increased awareness regarding sanitation and the introduction of vaccines, malaria eradication remains elusive due to the absence of a complete eradication technique [4]. The *Plasmodium falciparum*, as one of the causative agents of malaria, requires two hosts, a vertebrate and a mosquito. The infected Anopheles mosquito transmits the parasites (sporozoites) into the bloodstream. The sporozoites then travel to the liver and infiltrate parenchymal cells, where they multiply and transform into tissue schizonts. This marks the primary phase of infection, known as the pre-erythrocytic or primary erythrocytic phase, which varies in duration (5–16 days) depending on the Plasmodium species. Upon rupture of the tissue schizonts, numerous merozoites are released into the bloodstream. These merozoites initiate the erythrocytic phase or blood cycle by invading and infecting red blood cells [4]. In the secondary exoerythrocytic phase, a subset of these parasites invades additional liver cells [5]. By comprehending and targeting each stage of this intricate life cycle, the eradication of the disease can be effectively pursued.

Infections caused by *P. ovale* and *P. vivax* may harbor dormant tissue parasites known as hypnozoites, whereas *P. falciparum* and *P. malariae* infections do not exhibit this characteristic [6]. These dormant parasites have the potential to cause relapses in infected individuals for months or even years after the initial infection. Most parasites undergo asexual development within red blood cells, progressing from trophozoites to mature schizonts [7]. Upon rupture, the schizont-containing red blood cells release 6-32 merozoites, triggering symptoms such as chills and fever. These liberated merozoites go on to infect additional red blood cells, perpetuating the cycle. Some of these merozoites developed into male and female parasites called gametocytes. Anopheles mosquito ingests these gametocytes during a blood meal, initiating a sexual cycle called sporogony, resulting in the formation of zygotes. The zygotes invade the mosquito's midgut wall and mature into oocysts. As the oocysts grow, they rupture, releasing sporozoites that infect the mosquito's salivary glands. The introduction of these sporozoites into another human through mosquito bites initiates the malaria life cycle once again [6]. During its life cycle, the parasite experiences numerous cellular differentiation and metamorphoses, which require the breakdown of superfluous proteins and organelles within the host cells [8]. Understanding the intricacies of this degradation pathway may pave the way for the development of novel antimalarial drugs.

The role of traditional medicine remains significant in healthcare, with approximately 80 % of the global population relying on plant-based traditional healthcare products for primary medical needs [9]. The foundations of many existing antimalarial drugs can be traced back to natural product scaffolds. In the past, compounds like Quinine, artemisinin, and others were extracted from herbal remedies. [10, 11]. *Andrographis paniculata* plant, belonging to the Acanthaceae family, is renowned for its medicinal properties and often referred to as the "king of bitters". It is widely domiciled in tropical and subtropical regions of Asia and India. In different regions, it goes by various names such as "Kalmegh" in India, "Chuan-Xin-Lian" in China, "Fah Tha Lai" in Thailand, "Hempedu bumi" in Malaysia, "Senshinren" in Japan, and "green chiretta" in Scandinavia [11]. In Nigeria, it is known as 'Jogbo' due to its bitter taste, and among the Yoruba-speaking natives, it is popularly called 'Mejemeje' (seven-seven) because the typical dosage consists of seven raw leaves eaten once or twice daily for about five days. It is commonly used in the treatment of febrile illness or chronic debility. Some herbalists also recommend it for hypertension [12]. It is worth noting that *Andrographis paniculata* extracts have been associated with various medicinal properties, including antibacterial activities, derived from their traditional use. In Africa, a significant portion of the population still believes in and relies on herbal preparations for the treatment of diverse ailments, attesting to their perceived beneficial effects [13]. *A. paniculata* yields various phytochemicals, including alkaloids, terpenoids, cardiac glycosides, saponins, and flavonoids [14]. Notably, a study conducted by Megantra, [15] highlights the presence of andrographolide, a crucial compound found in *A. paniculata*. Andrographolide is classified as a diterpenoid molecule and has demonstrated inhibitory effects on a vital protease of malaria parasites. Additionally, other *in silico* studies conducted indicate that extracts derived from *A. paniculata* exhibit significant antiplasmodial activity against *P. falciparum* [16]. These findings shed light on the potential of *A. paniculata* as a valuable resource for the development of antimalarial treatments.

In recent years, the utilization of computational screening methods has emerged as a valuable technique in drug development, owing to its speed and cost-effectiveness [17]. Understanding the interaction between ligands and proteins serves as a rapid approach to identifying potential drug candidates and targets. Molecular docking, a computational modelling approach employed in the process of drug discovery, facilitates the prediction of potential interactions between two molecules, thereby generating binding energy [18]. This technique not only predicts the binding mechanism between small molecules and macromolecules (protein-ligand docking) but also between two macromolecules i.e., protein-protein docking [19].

By employing computational approaches like molecular docking and molecular dynamic simulation, researchers can expedite the identification of promising compounds and their interactions with target proteins, aiding in the efficient discovery and development of new drugs. The importance of designing drugs using the binding affinity and selectivity of target molecules has grown significantly. Therefore, the capacity for biological screening and chemical synthesis has experienced a substantial boost due to the progress in computational biology [17].

Also, there is a growing need for early information regarding absorption, distribution, metabolism, excretion (ADME), and toxicity data, collectively referred to as ADMET data. Screening for Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) plays a crucial role in selecting the promising compound [20]. ADMET involves comprehending the reactions and fate of pharmaceutical compounds inside an organism, specifically within the human body. Poor ADMET characteristics can lead to the failure of drug development projects at the stage of clinical trials. Hence, obtaining ADMET properties during the early stages of drug discovery is recommended [21]. By considering ADMET factors early on, researchers can mitigate potential issues and enhance the success rate of drug development endeavours, ensuring the development of safe and effective medications.

In this study, *in silico* approaches are used to predict valuable inhibitory compounds from *A. paniculata* against the validated drug targets of different stages of *P. falciparum*, which could aid the development of novel and effective drugs for the treatment, control and prevention of malaria.

Materials and methods

Collection and processing of plant

The fresh aerial part of *A. paniculata* plants was gathered from Olorunda Abaa, Akobo, Ibadan, and subsequently verified for its

authenticity at the herbarium of the Department of Botany, University of Ibadan and Federal Research Institute of Ibadan, Jericho, Ibadan. After collection, the plant materials were subjected to air-drying for a duration of two weeks under normal room temperature conditions. Once completely dried, the plants were pulverized into a fine powder and carefully stored in a tightly sealed container. Proper labeling was applied to the bottle, and it was placed in a cupboard for safekeeping.

Extraction of plant material

To extract the plant constituents, it was immersed in a solution comprising of 250 mL of a mixture containing dichloromethane (DCM) and methanol in a 1: 1 ratio. This soaking process took place at room temperature for a duration of 48 h, during which the mixture was consistently agitated. Subsequently, a rotary evaporator was employed to filter and eliminate the solvent under vacuum conditions, resulting in a brown extract weighing 30 g. The obtained extract was then stored in a properly labeled bottle for further use.

Qualitative and quantitative phytochemical analysis

Gas Chromatography provides excellent resolution in separating volatile and semi-volatile compounds; however, it does not possess the capability to identify them. Mass Spectrophotometry was employed to identify and quantify these compounds. This analytical technique offers comprehensive structural information for the majority of compounds, enabling accurate identification and quantification.

All chromatographic separations were performed on capillary columns with the specifications: length, 30 m, internal diameter 0.2 um, thickness, 250 um, and treatment with phenyl methyl silox. Other GC-MS conditions are ion source temperature (EI), 250 °C, interface temperature; 300 °C, pressure; 16.2 psi, out time, 1.8 mm, 1 µL injector in Split mode with split ratio 1: 50 with injection temperature of 300 °C the column temperature started at 35 °C for 5 mins and changed to 150 °C at the rate of 40 °C/min. The temperature was raised to 250 °C at the rate of 20 °C/min and held for 5 min. The total elution was 47.5 minutes. Ms Solution software provided by the supplier was used to control the system and to acquire the data. Identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from the NIST library (NISTII).

Preparation of target proteins

The Protein Data Bank (PDB) (http://rcsb.org) was utilized to retrieve the three-dimensional (3D) structures of the selected drug targets [22]. To facilitate the docking process, the drug targets underwent necessary processing to ensure they were in the appropriate format for uploading onto SwissDock. To refine the proteins for the docking study, certain steps were taken. Firstly, hydrogen atoms were added, and non-essential water molecules and heteroatoms were eliminated from the target protein structures. This refinement process was carried out using Biovia Discovery Studio Visualizer [23] and the resulting structures were saved in PDB format.

Subsequently, the processed protein structures were converted to macromolecules utilizing the PyRx tool [24] and selecting the “make macromolecule” option. The default configuration parameters were applied, and any missing amino acids in the target were all added. Finally, the region of the protein structure recognized as the potential ligand binding domains was identified for the docking studies.

Determination of active sites of proteins

The identification of amino acids within the active sites of the targets was accomplished using the Computed Atlas for Surface Topography of Proteins (CASTp). Available at http://sts.bioe.uic.edu/castp/index.html.

Ligand preparation for docking

For the purpose of docking studies, the three-dimensional structure (3D) of the phytocompounds were acquired from the PubChem database. PubChem, accessible at https://pubchem.ncbi.nlm.nih.gov, serves as a comprehensive general repository that offers information on chemical substances and their associated biological activities [25]. The optimization of ligands was performed using Universal Force Field (UFF) energy minimization parameter, employing a conjugate gradient optimization algorithm for a total of 200 steps. Energy minimization was executed within PyRx [26] using the open babel tool to achieve the lowest free energy state. Subsequently, the optimized ligands were converted into PDBQT formats, which are suitable for molecular docking analysis.

Absorption, distribution, metabolism, excretion and toxicity (ADMET) properties

The early-phase prediction of ADMET properties plays a crucial role in eliminating potential drug molecule failures and prioritizing promising drug candidates during the process of drug development. These ADMET studies serve as a viable alternative to traditional *in vitro* and *in vivo* experiments. By enabling the early prediction of these properties, substantial cost reductions can be achieved within the realm of drug research [27]. The ligands’ SMILES format was utilized to submit them to both admetSAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2/) and admetLab servers (ADMETlab 2.0 (https://admetmesh.scbdd.com/). This allowed for an examination of their drug-likeness as well as various pharmacokinetics and pharmacodynamics parameters. The admetSAR and admetLab tools provided the ADMET properties of the target compounds, while additional calculations were performed for properties such as Blood-Brain Barrier (BBB) penetration, Human Intestinal Absorption (HIA), P-glycoprotein interaction, AMES evaluation, carcinogenicity assessment, Human Oral Bioavailability (HOB), and $CaCO_2$ predictions.

Molecular docking

The interaction and the binding efficiency of the protein targets of the four stages of *P. falciparum* (Table 1): PFCDPK4 (4qox) (Calcium-independent protein kinases), PFDHR-TS (IJ31) (*P. falciparum* dihydrofolate reductase-thymidylate synthase), PFPM1 (2R9B) (*P. falciparum* proplasmepsin 1), PFPNP (5ZNC) (*P. falciparum* purine nucleoside phosphorylase), PFS48/45 (6e63) (*Plasmodium falciparum* gametocyte surface protein), PFS230 (7jum) (*P. falciparum* cysteine-rich 230 kDa gamete surface protein), PFPK7 (2pml) (*Plasmodium falciparum* protein kinase 7), PFA-M1 (4zw5) (*Plasmodium falciparum* M1 alanyl aminopeptidase and M17 (leucyl aminopeptidase complex) and the potential active ingredients of *A. paniculata* was investigated using the Autodock PyRx docking tool [27]. The SDF (structure-data file) structures of the phytoconstituents and standard ligands i.e., Proguanil, Artenimol, Halofantine, Quinine, Ganoplacide, Cipargamin, Tafenoquine, and Primaquine were obtained from the PubChem database. The docked ligand-receptor complex was examined on the lowest binding energy (kcal/mol) values. The molecular docking analysis was carried out using Autodock Vina from PyRx. Molecular interactions between proteins and ligands were viewed with Chimera 1.14 and discovery studio 2021 [28] for the best docked poses [25]. The interaction analysis involves studying non-covalent interactions, such as hydrogen bonds, charge interactions, hydrophobic interactions, Pi-stacking, and van der Waals binding forces, based on the docking score of ligands against the binding target and a commercially used drug [29].

Molecular dynamics simulations (MDS)

Molecular dynamics (MD) simulation of protein–ligand complexes was examined by using WebGRO. The MD simulation was performed to gain insight into the structural stability, residual fluctuation, compactness of the structure, and binding affinity. These aspects were assessed by measuring the backbone Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSFs) of all residues and the number of H-bonds. Using the WebGRO server, RMSD profiles of protein-ligand complexes were assessed. Using the GROMOS96 43a1 forcefield, the best-docked protein-ligand complexes were made ready for MD. The PRODRG tool was used to create the ligand topology [30].

Results

Active components and their selected targets

A total of forty-three (43) phytoconstituents were identified from *A. paniculata*, and eight proteins (two proteins for each stage) of *P. falciparum* life circle were involved. Eight standard drugs that has been proven and used to treat malaria were selected from drug bank as controls (Table 2).

Drug-likeness and ADMET profile

Out of the Forty-three phytoconstituents from *A.paniculata*, forty were predicted to score positive and three were negative. 8-Methylenecycl-ooctene-3,4-diol was predicted to score the highest druglikeness score of 2.915. The results are summarized in Table 2. The ADMET profile of each phytoconstituent, as predicted by ADMETLAB and Admetsar 2.0, is represented using the heat map, as shown in Figure 1. All the phytocompounds from *A. paniculata* are predicted to have positive human intestinal absorption scores (HIA). All the phytoconstituents except 1-(2-Methoxy-4-methyl-cyclohex-3-enyl)-1-methylethylamine, N-Ethyl-3-methoxy-4 methylphenethylamine, 2-Dimethylamino 2 'methoxyacetophenone, Azetidine, Octanoic acid, Cyclopentaneacetic, (7a-Isopropenyl-4,5 dimethyloctahydroinden-4-yl)methanol acid, Heptadecanal, Z-8-Methyl-9-tetradecenoic acid, 8-methyl-1-Undecene, butyl ester, Allyl n-octyl ether, cis-11-Octadecenoic acid, 14-Hydroxy-14-methyl-hexadec-15-enoic acid, cis-9-Hexadecenal, and Cyclopropaneoctanal are blood-brain barrier permeant (BBB). Also, all phytoconstituents apart from 2, 2-Bis (butoxy) propionic acid are predicted to be non-inhibitors of P-glycogen. From all the phytocompounds, none of them is an inhibitor of CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP1A2. None of phytoconstituents also show tendency towards mutagenic and only two phytocompounds are predicted to be carcinogenic (Thiodiglycol and 8-Methylenecycloocte-ne-3,4-diol) (Figure 1).

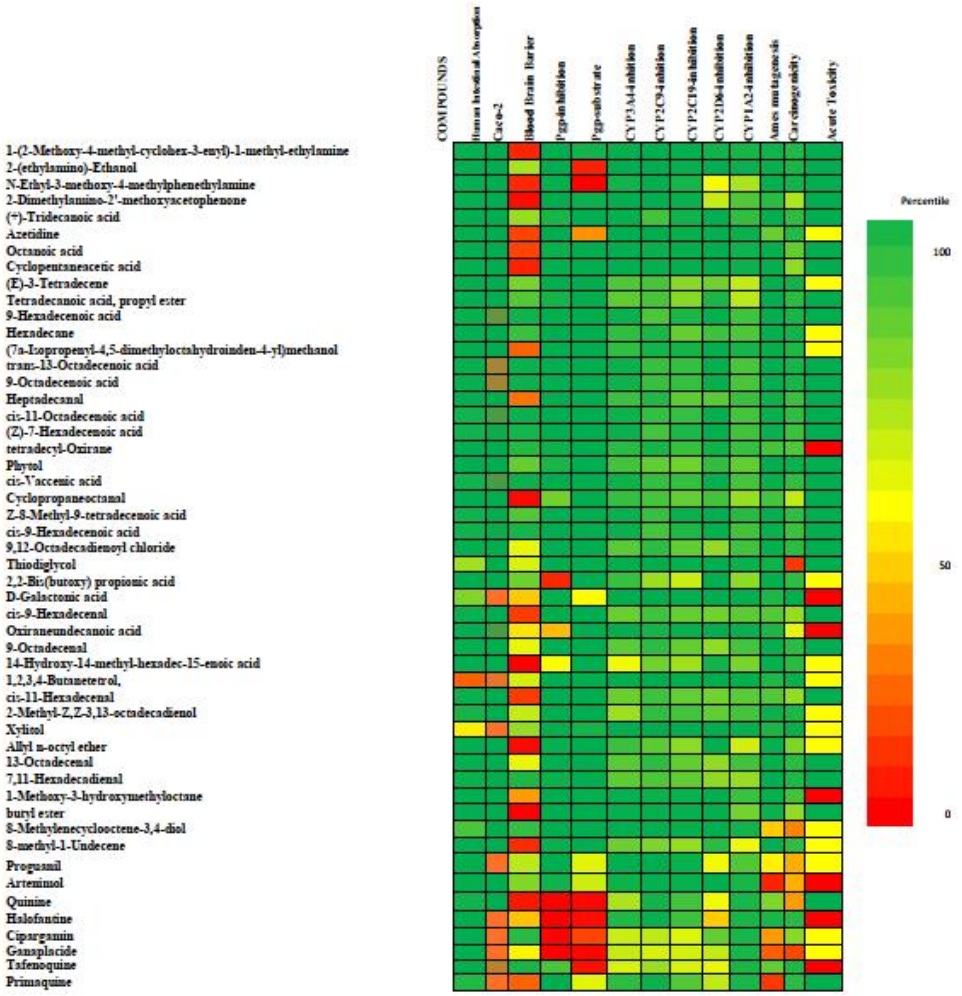

Figure 1 Heat Map of ADMET profile of phytocompounds of *A. paniculata*. The bad, average, and excellent phytoconstituents for each parameter are represented by red, yellow, and green colours, respectively.

Table 1 Malaria life cycle stages and their respective targets

Stages of life cycle	Targets	PDB Name
Asexual blood-stage	PFCDPK4	4qox
	PFDHR-TS	IJ31
Hepatic schizonts stage – liver prophylaxis	PFPM1	2R9B
	PFPNP	5ZNC
Transmission (targeting parasite gametocytes) – gametocyte transmission blocking	PFS48/45	6e63
	PFS230	7jum
Transmission (targeting the insect vector) – mosquitocidals	PFPK7	2pml
	PFA-M1	4zw5

Table 2 The molecular formula, physicochemical properties and drug-likeness scores of phytocompound from *A.paniculata*.

Compounds name	Molecular formula	Molecular mass g/mol	NHBA	NHBD	MolLogP	DLS
1-(2-Methoxy-4-methyl-cyclohex-3-enyl)-1-methyl-ethylamine	$C_{11}H_{21}NO$	183.29	2	2	1.713	2.119
2-(ethylamino)-Ethanol	$C_4H_{11}NO$	89.14	2	2	–0.448	0.382
N-Ethyl-3-methoxy-4-methylphenethylamine	$C_{12}H_{19}NO$	223.27	2	1	2.466	-0.351
2-Dimethylamino-2'-methoxyacetophenone	$C_{11}H_{15}NO_2$	193.28	3	0	1.448	-0.849
(+)-Tridecanoic acid	$C_{13}H_{26}O_2$	193.24	2	1	5.321	0.462
Allyl n-octyl ether	$C_{11}H_{22}O$	214.34	1	0	4.276	0.029
Azetidine	C_3H_7N	170.29	1	1	–0.358	0.11
Octanoic acid	$C_8H_{16}O_2$	57.09	2	1	2.85	0.694
Cyclopentaneacetic acid	$C_7H_{12}O_2$	144.21	2	1	1.926	0.246
1-Methoxy-3-hydroxymethyloctane	$C_{10}H_{22}O_2$	178.65	2	1	2.312	1.439
8-methyl-1-Undecene	$C_{12}H_{24}$	128.169	0	0	5.912	1.492
(E)-3-Tetradecene	$C_{14}H_{28}$	174.28	0	0	6.938	1.268
1,2,3,4-Butanetetrol	$C_4H_{10}O_4$	168.32	4	4	–2.024	1.196
Tetradecanoic acid	$C_{14}H_{28}O_2$	343.29	2	0	6.981	0.12
Xylitol	$C_5H_{12}O_5$	196.37	5	5	–2.238	1.323
2,2-Bis(butoxy) propionic acid	$C_{15}H_{30}O_4$	168.1	4	0	4.37	0.04
butyl ester	$C_6H_{12}O_2$	122.12	2	0	1.484	0.71
9-Hexadecenoic acid	$C_{16}H_{30}O_2$	122.19	2	1	5.95	0.966
cis-9-Hexadecenal	$C_{16}H_{30}O$	270.5	1	0	4.894	1.167
Hexadecane	$C_{16}H_{34}$	152.15	0	0	8.415	0.153
(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	$C_{15}H_{26}O$	274.4	1	1	3.567	2.714
8-Methylenecyclooctene-3,4-diol	$C_9H_{14}O_2$	116.16	2	2	0.797	2.915
trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	196.16	2	1	6.829	0.941
9-Octadecenoic acid	$C_{18}H_{34}O_2$	254.41	2	1	6.83	0.869
7,11-Hexadecadienal	$C_{16}H_{28}O$	238.41	1	0	4.845	1.445
Heptadecanal	$C_{17}H_{34}O$	720.9	1	0	6.903	0.77
Oxiraneundecanoic acid	$C_{13}H_{24}O_3$	226.44g	3	1	3.387	0.891
cis-11-Octadecenoic acid	$C_{18}H_{34}O_2$	268.39	2	1	6.169	0.869
(Z)-7-Hexadecenoic acid	$C_{16}H_{30}O_2$	222.37	2	1	5.184	0.966
13-Octadecenal	$C_{18}H_{34}O$	154.21	1	0	6.663	1.119
9-Octadecenal	$C_{18}H_{34}O$	282.5	1	0	6.665	1.044
tetradecyl-Oxirane	$C_{18}H_{34}O_2$	282.5	2	0	5.979	0.488
Phytol	$C_{20}H_{40}O$	236.39	1	1	7.385	1.532
14-Hydroxy-14-methyl-hexadec-15-enoic acid	$C_{18}H_{34}O_3$	254.5	3	1	5.332	1.116
cis-Vaccenic acid	$C_{18}H_{34}O_2$	228.33	2	1	6.169	0.869
Cyclopropaneoctanal	$C_{11}H_{20}O$	224.3	1	0	3.55	1.385
2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	282.5	1	1	5.367	1.506
cis-11-Hexadecenal	$C_{16}H_{30}O$	254.41	1	0	4.893	1.251
Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	266.5	2	1	4.593	1.795
cis-9-Hexadecenoic acid	$C_{16}H_{30}O_2$	266.5	2	1	5.184	0.966
9,12-Octadecadienoyl chloride	$C_{18}H_{31}ClO$	785.3	1	0	6.663	1.119
Thiodiglycol	$C_4H_{10}O_2S$	282.5	2	2	–0.514	–0.301

Molecular docking analysis

The phytoconstituents of *A. paniculata* were docked with the selected proteins of the four stages of *Plasmodium* lifecycle. Table 3 shows the best four binding energies of the docked compounds for each protein. Figures 2 (A, B, C, D) show the 3D and 2D (two-dimensional and three-dimensional) representations of the molecular binding interactions of the amino acid residues of the targets with the compounds predicted to have the lowest binding energies.

Molecular Dynamics Simulation Analysis

To evaluate the stability of the protein-ligand complexes at their binding sites, molecular dynamics (MD) simulation was conducted. The entire simulation was carried out for 50 nanoseconds in the production phase for the protein-ligand complexes. The stability of the

complexes during the MD simulation was evaluated using two parameters: Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSFs). Figure 3–10 shows the RMSDs and RMSFs of the proteins when compared to their initial positions.

Discussion

Consistent endeavours have been undertaken to eliminate malaria over many decades [31]. The primary emphasis of research has been directed towards discovering strategies to hinder the development and elimination of resistance against existing medications, as this poses the most significant challenge to the effectiveness of antimalarial treatment. However, there has been relatively less emphasis on the development of innovative drugs that are effective against all stages of the *P. falciparum* life cycle, despite the urgent requirement for such medications.

In recent decades, pharmaceutical researchers have dedicated their efforts extensively to exploring the various stages of the plasmodium life cycle to identify and utilize new therapeutic targets derived from indigenous plants [32]. Additionally, ongoing research is being conducted to create innovative chemical compounds or targeted inhibitors that can effectively act on these newly discovered targets. The extraction of phytochemicals from medicinal plants is significantly affected by the selection of the solvent used for extraction and the methodology employed [33]. The reason for this is that medicinal plants consist of a wide range of chemical compounds, each with its polarity and chemical characteristics, resulting in solubility or insolubility in particular solvents [34].

In this study, methanol and dichloromethane were used as solvents for organic extraction, selected based on their wide polarities [35]. Methanol, being a polar solvent, was utilized to extract polar active chemicals from the plant material, whereas dichloromethane (DCM), a nonpolar solvent, was employed to extract nonpolar active compounds. Previous research findings revealed that *A. paniculata*

Table 3 Docking results of *plasmodium falciparum* molecular drug target.

Phytocompounds	Binding Affinity (Kcal/mol)	Compounds	Binding Affinity (Kcal/mol)
A			
IJ31		4QOS	
(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	–6.8	Phytol	–6.2
2-Methyl-Z,Z-3,13-octadecadienol	–6.3	9-Octadecenoic acid	–6.2
N-Ethyl-3-methoxy-4 methylphenethylamine	–6.1	N-Ethyl-3-methoxy-4-methylphenethylamine	–6.2
9,12-Octadecadienoyl chloride	–6.0	(Z)-7-Hexadecenoic acid	–6.1
Proguanil	–5.5	Artenimol	–8.1
B			
2R9B		5ZNC	
(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-y l)methanol	–6.1	N-Ethyl-3-methoxy-4-methylphenethylamine	–6.5
cis-Vaccenic acid	–6.0	cis-Vaccenic acid	–6.4
14-Hydroxy-14-methyl-hexadec-15-enoic acid	–6.0	Phytol	–6.0
9,12-Octadecadienoyl chloride	–6.0	cis-11-Octadecenoic acid	–6.0
Halofantine	–7.6	Quinine	–6.4
C			
6E63		7JUM	
N-Ethyl-3-methoxy-4-methylphenethylamine	–5.7	(7a-Isopropenyl-4,5-dimethyloctahydroinde n-4-yl)methanol	–5.7
2-Dimethylamino-2'-methoxyacetophenone	–5.6	D-Galactonic acid	–5.6
9,12-Octadecadienoyl chloride	–5.5	N-Ethyl-3-methoxy-4-methylphenethylamine	–5.1
(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-y l)methanol	–5.4	cis-11-Octadecenoic acid	–5.1
Ganoplacide	–0.3	Cipargamin	–7.3
D			
2pml		4zw5	
(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-y l)methanol	–6.8	(7a-Isopropenyl-4,5-dimethyloctahydroinde n-4-yl)methanol	–6.8
9,12-Octadecadienoyl chloride	–6.5	Phytol	–6.4
cis-Vaccenic acid	–6.3	cis-Vaccenic acid	–6.3
Phytol	–6.2	2-Dimethylamino-2'-methoxyacetophenone	–6.1
Tafenoquine	–8.7	Primaquine	–6.8

Note: Binding energies of the top docked compounds for each protein of each four stages of *Plasmodium* lifecycle. A: Asexual blood-stage. B: Hepatic schizonts stage of *Plasmodium falciparum*. C: gametocyte transmission stage of *Plasmodium falciparum*. D: the transmission stage of *Plasmodium falciparum*.

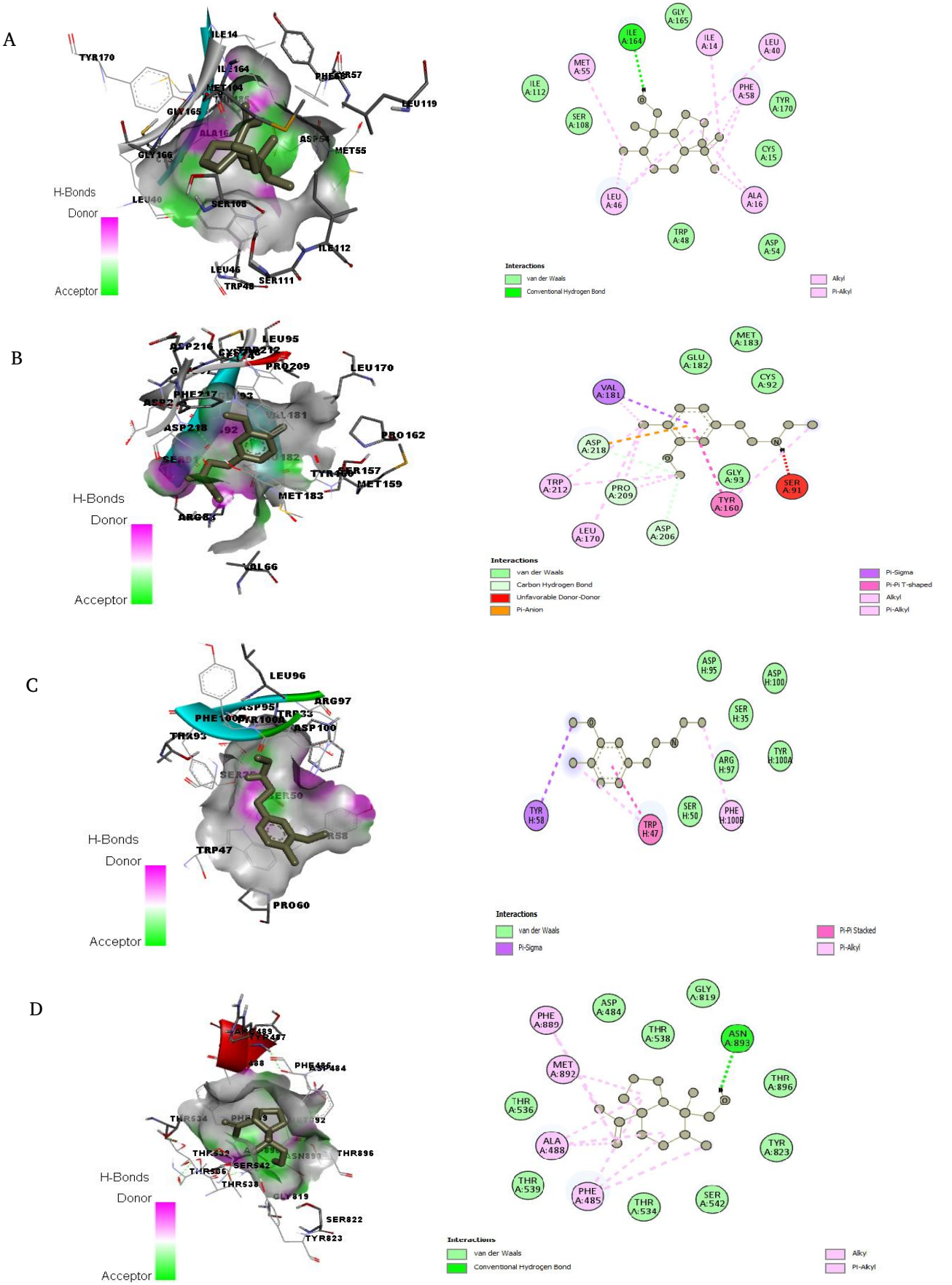

Figure 2 Molecular interactions between amino acid residues of the various targets and phytocompound. (A) IJ31 and (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl) methanol (B) 5ZNC and N-Ethyl-3-methoxy-4-methylphenethylamine (C) 6E63 and N-Ethyl-3-methoxy-4-methylphenethylamine (D) 4zw5 and (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl) methanol.

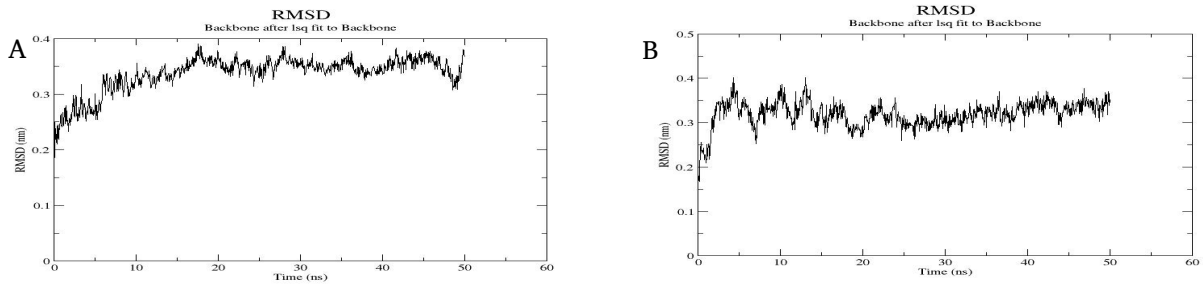

Figure 3 A: RMSD values: 1J3I- (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl) methanol. B: 1J3I- Proguanil complex with 50 ns, molecular dynamic simulation.

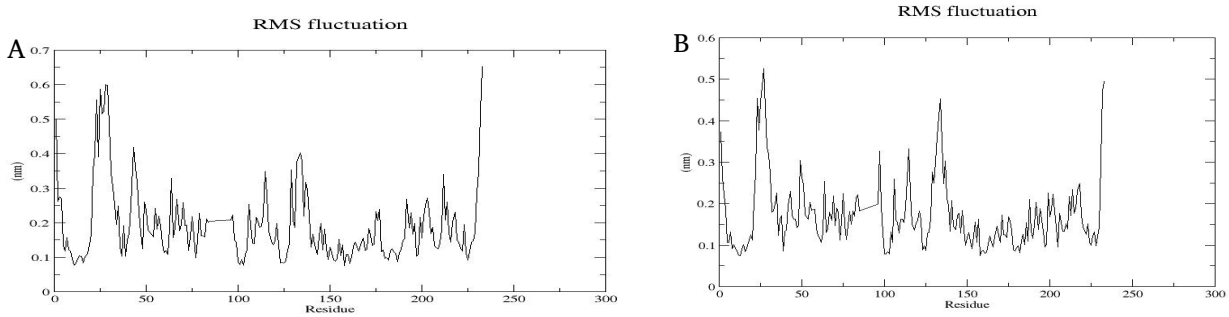

Figure 4 A: RMSF values: 1J3I- (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl) methanol. B: 1J3I- Proguanil complex with 50 ns molecular dynamic simulation.

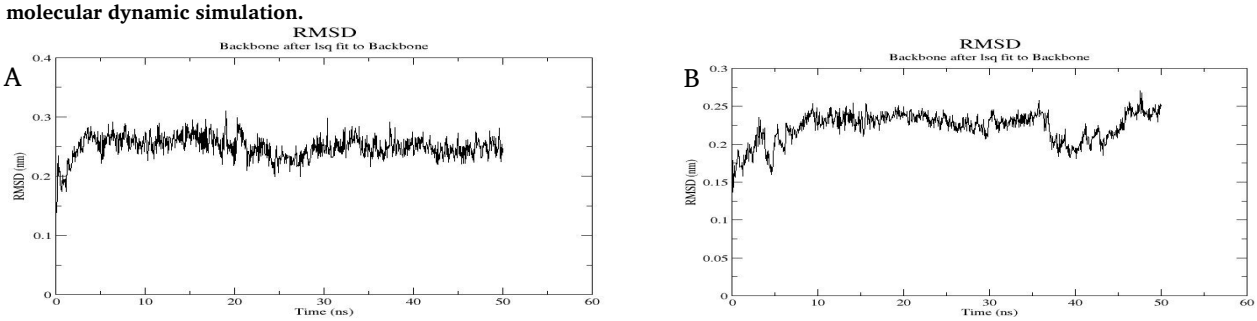

Figure 5 A: RMSD values: 5ZNC- N-Ethyl-3-methoxy-4-methylphenethylamine. B: 5ZNC- Quinine complex with 50 ns MD simulation

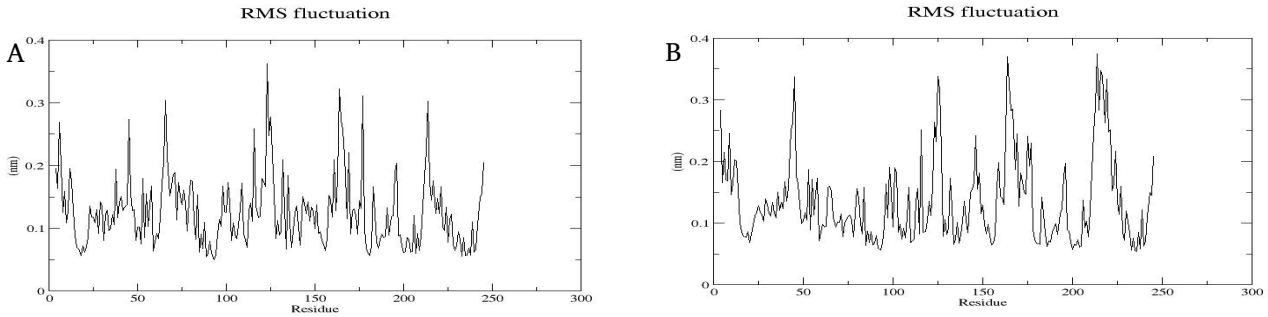

Figure 6 A: RMSF values: 5ZNC- N-Ethyl-3-methoxy-4-methylphenethylamine. B: 5ZNC- Quinine complex with 50 ns molecular dynamic simulation.

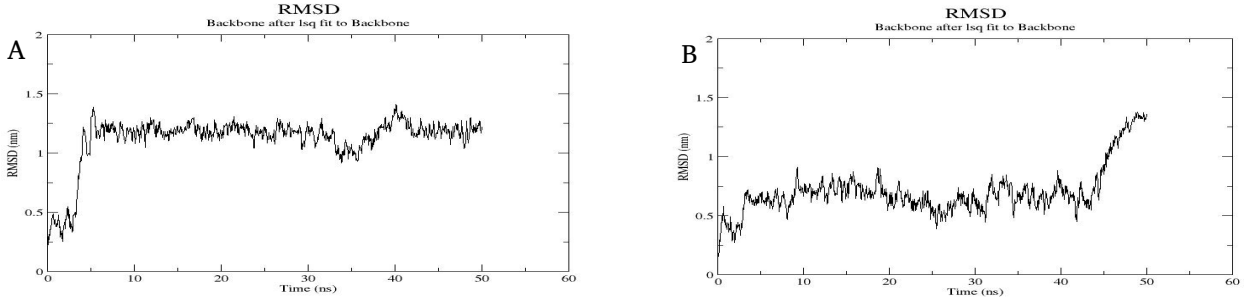

Figure 7 A: RMSD values: 6E63 - N-Ethyl-3-methoxy-4-methylphenethylamine. B: 6E63 - Ganoplacide complex with 50 molecular dynamic simulation.

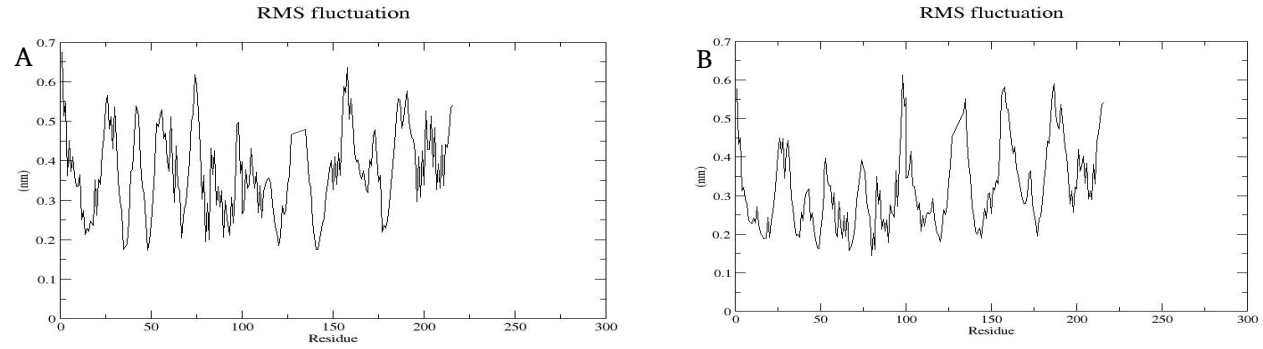

Figure 8 A: RMSF values: 6E63 - N-Ethyl-3-methoxy-4-methylphenethylamine. B: 6E63 - Ganoplacide complex with 50 ns molecular dynamic simulation.

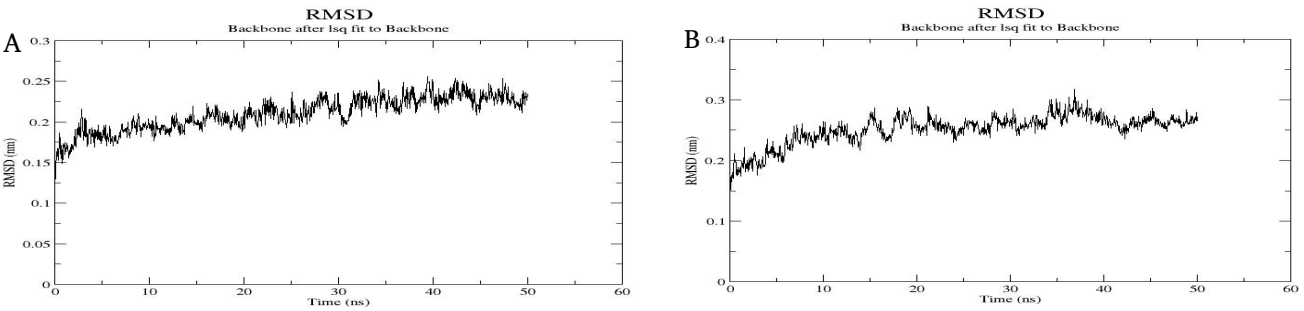

Figure 9 A: RMSD: 4zw5 - (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl) methanol. B: 4zw5 - Primaquine complex with 50 ns MD simulation.

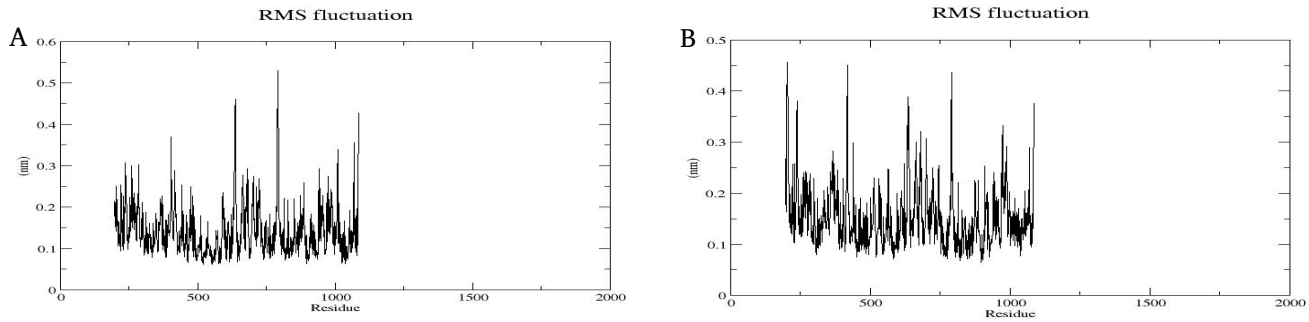

Figure 10 A: RMSF: 4zw5 - (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl) methanol. B: 4zw5 - Primaquine complex with 50 ns MD simulation.

extracts prepared with DCM: methanol (1: 1) exhibited superior effectiveness against the Plasmodium parasite compared to aqueous extracts [34, 36].

Some Antifolates such as pyrimidine and cycloguanil are active-site inhibitors of the malaria dihydrofolate reductase (DHFR) enzyme and have been successfully used to treat *P. falciparum malaria* [37]. These DHFR are encoded as two discrete enzymes, the malaria DHFR is encoded on the polypeptide chain as the thymidylate synthase (TS) enzyme, which catalyzes the upstream reaction of converting methylene tetrahydrofolate (CH2H4-folate) to H2-folate. This bifunctional thymidylate synthase-dihydro folate reductase (TS-DHFR) enzyme is the target of antifolate drug design in *P. falciparum* [32].

From this study, it was observed that (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl) methanol (–6.8) compound had a better binding affinity with IJ31 (DHFR-TS) than the standard drug proguanil (–5.5) in the asexual blood stage of *Plasmodium falciparum*. Moreover, a higher binding affinity depends on both the number of hydrogen bonds established and the ligands' capacity to form more hydrophobic interactions with the hydrophobic amino acids near the ligand's binding site [38]. To validate the outcomes of molecular docking, further computational exercise with additional accurate molecular dynamics simulation was performed on the docked complexes. It was observed that (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl) methanol is more stable throughout the simulation period than the control drug.

The enzyme purine nucleoside phosphorylase (PfPNP) from *P. falciparum*, involved in purine salvage, is a promising candidate for drug targeting. Earlier investigations, utilizing transition state analogue inhibitors against PfPNP, demonstrated their efficacy in killing *P. falciparum in vitro* [39]. Unlike most mammalian cells, erythrocytes lack the biochemical machinery for de novo purine synthesis but serve as an abundant source of purine salvage enzymes which is a major asexual cycle mechanism for malaria parasite, notably purine nucleoside phosphorylase (PNP) and adenosine deaminase (ADA) [40]. Plasmodium's purine salvage pathway initiates with the deamination of adenosine to inosine catalysed by ADA, subsequently transforming inosine into hypoxanthine through the action of PNP. The ultimate enzyme in this pathway is hypoxanthine-guanine-xanthine phosphoribosyltransferase(HGXPRT). Hypoxanthine serves as a precursor for all purines and holds a pivotal role in nucleic acid synthesis in *P. falciparum* [41]. Intriguingly, *P. falciparum* exhibits the ability to thrive in erythrocytes lacking either PNP or ADA, implying that the enzymes PfADA and PfPNP are adequate for the parasite's survival within erythrocytes [42]. In this study, N-Ethyl-3-methoxy-4-methylphenethylamine showed the lowest binding energy among the group of phytoconstituents docked against PfPNP (5ZNC) in the Hepatic schizonts stage of *P. falciparum*.

We further noticed that N-Ethyl-3-methoxy-4-methylphenethylamine has a lower binding energy and is more stable throughout the simulation period than the control drug, Quinine. This inhibitory effect of N-Ethyl-3-methoxy-4-methylphenethylamine is in line with previous studies which identified it as a compound with antiplasmodial effect [43]. Also, N-Ethyl-3-methoxy-4 methylphenethylamine has a lower binding energy with stronger stability than the standard drug Ganoplacide against PFS48/45 of gametocyte transmission blocking stage throughout the simulation period as a result of the strong interaction it poses with the protein predicting it as a good inhibitor.

Malarial neutral aminopeptidases (Plasmodium falciparum M1 alanyl aminopeptidase and M17) play a crucial role in the final phases of hemoglobin digestion [44]. They are essential for supplying amino acids necessary for the growth and development of the parasite within the erythrocyte [44]. In this study, (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl) methanol was shown to have the same lowest binding energy with the control (Primaquine) against the 4zw5 targeting the insect vector transmission stage but confer a better stability in the protein-ligand complex throughout the simulation period than the control.

The pharmacokinetic profile of a drug molecule, encompassing its ADMET characteristics, plays a crucial role in determining its pharmacodynamic activities [45]. The drug-like properties of individual phytoconstituents from *A. paniculata* were assessed using "Lipinski's Rule of Five", a qualitative evaluation that considers factors such as oral bioavailability, to predict their drug-likeness characteristics (Lipinski, 2004). In this study, a significant number of compounds exhibited favorable drug-likeness scores, indicating their potential for oral bioavailability. Additionally, the ADMET properties of each phytoconstituent were assessed to evaluate crucial pharmacokinetic parameters in the human body, including molecule-specific toxicity, and were compared to the control group.

Conclusion

Plant-based phytoconstituents have proven beneficial and are crucial in the creation of new antimalarial drugs. Considering the ethnobotanical and experimental claims of the use of *Andrographic paniculata* as antimalarial, the various phytocompounds derived from this plant confirm these claims. This *in silico* study, which involved molecular docking and molecular dynamic simulation, predicted and provided information on the compounds of *A. paniculata,* which show a strong inhibitory potential against 4 different life stages of *Plasmodium falciparum* validated drug targets (IJ31, 5ZNC, 6E63, 4zw5), especially 7a-Isopropenyl-4, 5-dimethyloctahydroinden-4yl) - Methanol and N-Ethyl-3-methoxy-4-methylphenethylamine on the asexual blood stage and gametocyte (sporozoites) transmission stage and Hepatic schizont (liver prophylaxis) and gametocyte (meroziotes) transmission stages, respectively.

Thus, these bioactive compounds of *A. paniculata* have strong potential and can be developed as antiplasmodial agents. These promising inhibitors merit further *in vivo* biological investigation and pharmacological work as potential therapeutic candidates. This can help confirm these claims and help develop an effective therapy against malaria.

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