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Unveiling Natures Arsenal: Harnessing *Entada africana's* Methanol Bark Extract to Combat Malarial infection Through Heme Polymerase Inhibition

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Abstract

Plasmodium parasites, which cause malaria, continue to pose a serious threat to global health, necessitating the continuous search for novel antimalarial agents. *Entada africana* is a plant known for its ethnomedicinal uses in treating various ailments associated with inflammation including malaria. Due to its reported antiplasmodial potentials, we studied the effect of the methanol bark extract of the plant. HPLC chromatogram of the methanol bark extract showed the presence of eight phyto-compounds namely coumaric acid, gallic acid, catechin, ferulic acid, quercetin, apigenin, rutin, and kaemferol. Thus, the study aimed at evaluating the antiplasmodial potential of methanol bark extract of *Entada africana* (MBEEA) through heme polymerase inhibition via *in silico* approaches. The *in silico* studies showed favourable binding affinities and stable interactions with heme polymerase, with rutin (-9.9 kcal/mol), apigenin (-8.0 kcal/mol) and catechin (-7.8 kcal/mol) having higher binding affinities compared to the standard drug, chloroquine (-6.7 kcal/mol). Hydrogen bond analysis reveals that ferulic acid (Asp 77, Ile 73 and Ala 29) and kaemferol (Arg 40, Arg 27 and Leu 74) form three hydrogen bonds. On the other hand, compounds like coumaric acid (Ala 291 and Arg 27), gallic acid (Ala 29 and Ser 76), catechin (Arg 40 and Arg 40), and quercetin (Ser 76 and Ser 76) form two hydrogen bonds with the amino acid residues, rutin forms two hydrogen bonds with Ser 76 and Leu 74, while apigenin forms one hydrogen bond with Arg 27 when compared to the standard drug, chloroquine (-6.7 kcal/mol) which forms no hydrogen bonds with the amino acid residues. Based on their pharmacokinetic characteristics, safety profiles, and appropriate drug-like ability, seven compounds were shown to have antiplasmodial properties by computational ADMET tests.

Keywords: Anti-plasmodial potential, binding affinities, chloroquine, heme polymerase inhibition, in silico studies, MBEEA, phyto-compounds

Introduction

Malaria has afflicted humanity for centuries, persisting as one of the most severe parasitic infectious diseases globally. Endemic to 85 countries and territories, malaria's toll is staggering, with 241 million cases and 627,000 deaths reported by the World Health Organization (WHO) in 2020, marking a concerning rise from 2019 [11]. Particularly devastating in impoverished regions, malaria, caused by *Plasmodium spp*., predominantly *Plasmodium falciparum* and *Plasmodium vivax*, spreads via infected *Anopheles* mosquitoes (24; 2). Following transmission, the parasite invades hepatocytes, proliferates within red blood cells, and triggers clinical symptoms, including fever, upon erythrocyte rupture [20, 27].

Despite numerous antiplasmodial agents, drug resistance poses a formidable challenge, especially in Africa, where favourable climatic conditions facilitate malaria transmission. WHO reported 405,000 malaria-related deaths in 2019, significantly impacting Africa's economy, with Nigeria alone contributing to over 23% of global cases [25]. In response, many Africans turn to indigenous remedies due to cost and accessibility concerns, highlighting the urgent need for alternative treatments.

Heme polymerase, an enzyme crucial for *Plasmodium species'* defense against heme toxicity, is a promising target for antimalarial intervention. Chloroquine, among other drugs, inhibits this enzyme's activity, emphasizing its therapeutic potential. *Entada africana*, a medicinal plant native to several African countries, including Nigeria, boasts traditional uses in treating inflammatory ailments, including malaria [12]. Notably, its methanol extract exhibits antioxidant, antimicrobial, anti-inflammatory, and antiplasmodial properties [7, 14, 15, 19].

This study aims to screen phytochemicals isolated from *Entada africana's* methanol bark extract via HPLC and evaluate their interactions with the parasite's heme polymerase using computational methods, including molecular docking, hydrogen bond analysis, 3D interaction mapping, and pharmacophore modeling. By identifying potential lead compounds that inhibit heme polymerase, this research seeks to disrupt hemozoin formation, thereby impairing the parasite's survival post-erythrocyte degradation. Leveraging *in silico* approaches in drug discovery and natural product research emerged as a valuable strategy in recent years [21].

Materials

Collection of Plant Materials and Authentication

Barks of *Entada africana* (Fabaceae) plant were collected from a local farm in Iwaro-Oka, Akoko South West Local Government Area. Latitude 7.278PN and longitude 5.1167°E, Ondo State, Nigeria. The plant materials were then identified by Dr. O. Obembe, and authenticated at Plant Science and Biotechnology Departmental Herbarium (PSBH), Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Plant voucher specimen designated as PSB-479 for the *Entada africana*. A bark sample was later deposited at the Herbarium.

Preparation of Methanol Bark Extract of *Entada Africana*

Fresh barks of *Entada africana* were harvested, stored and washed properly to remove debris and dust particles. The plant materials were air dried at room temperature for 3 weeks and grinded to fine powder using electric blender. The ground particles were soaked in aqueous methanol (1:4 w/v) for 72 hours with intermittent stirring after which it was filtered. The crude extract is concentrated under reduced pressure using rotary evaporator to remove the methanol solvent. This results in a thick, concentrated crude extract.

HPLC analysis

Sample Preparation for HPLC analysis

The Reversed phase chromatography HPLC system (Agilent Technologies 1200 HPLC, CA, USA), consisted of a horizontal flow-through planar centrifuge with a multilayer coil, a pump (JASCO, 880-PU), a microflow pH sensor (Broadley-James, Model 14, CA, USA), a manual injection valve with a 10 ml loop, and a fraction collector (JASCO, SF-212N). The upper phase, consisting of Hypersil BDS C_{18} (Agilent), was used as the stationary phase, while the lower phase was as the mobile phase. 100 mg of the plant extract (MBEEA) was taken into a conical flask and dissolved in 5 ml of HPLC grade methanol, a mixture of the stationary phase: mobile phase (3:1 v/v), and introduced through the injection port. The mobile phase which constitute of 0.1% formic acid + acetonitrile was pumped at 0.6 ml/min. A multi-wavelength detector (Waters, 490E) monitored the absorbance of the effluent at 280 nm. HPLC was performed according to the following chromatographic conditions as illustrated in Table 2.1.

Table 2.1: HPLC chromatographic conditions

Parameters	Settings
Agilent Technologies 1200 HPLC	Reversed phase chromatography
Mobile phase composition	0.1% formic acid + Acetonitrile
Stationary phase	Hypersil BDS C_{18} (Agilent)
Column Dimension	250mm x 4.0 mm
Flow rate	0.6 ml/min
Injection volume	10 µL
Detector wave length	280 nm
Mode of elution	Gradient elution

Ligand and Protein Preparation

The structure data file (sdf) format of eight (8) compounds from *Entada africana* gotten from HPLC analysis were downloaded from pubchem database (https://pubchem.ncbi.nlm.nih.gov) in sdf and imported into the workspace of Schrödinger suite interface (2017v1). The Ligprep tool of Schrödinger suite (2017v1) software were used to prepare the ligand for docking by optimizing their 3D structure and generating multiple conformers (shapes) for each compounds. This preparation process followed the method described by Omoboyowa *et al.* (16) which is a published protocol for preparing compounds for molecular docking.

Homology Modelling of Heme Polymerase and the Template Sequence Alignment

The experimental crystal structure of heme polymerase is not available in the protein data bank (PDB); hence, its 3D structure was modelled. The protein ID of the target, heme polymerase was retrieved from UniProt Knowledgebase (UniProtKB) with the accession number P09601. Afterwards, the protein ID was submitted to SWISS-MODEL25 web server to develop a model with sufficient query sequence coverage and sequence identity. The most reliable 3D structure was selected based on the Global Model Quality Estimation (GMQE) 26 and Qualitative Model Energy Analysis (QMEAN) 27 values. The similarity identity sequence between the amino acid sequences of the homology model of heme polymerase and the template structure (3 CYV) used for the homology model were confirmed using Clustal Omega version 1.2.1.29.

Hits Virtually Screening with E-Pharmacophore Model

The ligand (chloroquine) was used to generate an energy-optimized pharmacophore model which serves as a blueprint of the chemical features a molecule needs to bind to the crystal structure of heme polymerase. The model was generated from protein-ligand option of the phase develop pharmacophore tool of Schrödinger Suite (2017v1) [16]. The virtual screening base on E-pharmacophore was performed with the eight (8) hit compounds from the molecular docking scores. The hit compounds were prepared using Macro Model Minimization to optimize their 3D structure and the pharmacophore-based analysis was carried out with Phase module to generate a subset of molecules having chemical features for binding to heme polymerase according to the generated model. The fitness scores were used to justify the best hits.

Generation of QSAR Model

The FASTA sequence of the experimental inhibitory datasets for heme polymerase was downloaded from (www.ebi.ac.uk/chembl). Bioactive compounds with inhibitory activities against the target were obtained with their pIC_{50}, these datasets were converted to SDF format using Data-warrior, version 2 [16]. The SDF file was uploaded on the workspace of Maestro - Schrödinger Suite (2017v1) and prepared using Macro Model Minimization. Base on the pIC_{50} of the corresponding active compounds, the quantitative structural activity relationship (QSAR) model of the target was generated. The top predicting ranked model (kpls_molprint-2D_3) was selected. This model was utilized in predicting the pIC_{50} of the eight (8) hits from the molecular docking study.

Molecular Docking Analysis

Eight (8) phytocompounds namely; coumaric acid, gallic acid, catechin, ferulic acid, quercetin, apigenin. rutin and kaemferol were identified and screened by optimizing geometry and generating PDBQT files. The protein target (heme polymerase) was prepared by removing water and ions. Then, the resulting collection of potential ligands was docked into the heme polymerase. Hydrogen atoms were added to all proteins, and partial atomic charges were calculated setting up the docking run by selecting the protein target and phytochemical library, defining docking parameters such as grid size and energy range using AutoDock Vina in PyRx 30.8 (virtual screening tools). Initially, flexible-ligand docking was done. The grid box size was set to $35 \times 35 \times 35$ points with a spacing of 0.375 Å. For the calculation, 150 runs of the Lamarckian genetic algorithm (LGA) with 25 000 000 evaluations and 270 000 generations were performed.

Pharmacokinetics and Drug-Likeness Study (ADME/Tox)

The drug-likeness properties, pharmacokinetic profiles and Lipinski's violation analysis of the hits from *E. africana* were predicted with QIKPROP tool of Schrödinger Suite (2017v1) [17].

Statistical Analysis

The data was presented as mean ± SEM. A one-way ANOVA followed by Tukey's test were used to analyze the significant difference, where $p \leq 0.05$ was presented as statistical difference.

Software

In this study, Schrödinger software (2017v1), Data-warrior, version 2 and Clustal Omega version 1.2.1.29 were used as the computational tools.

Results

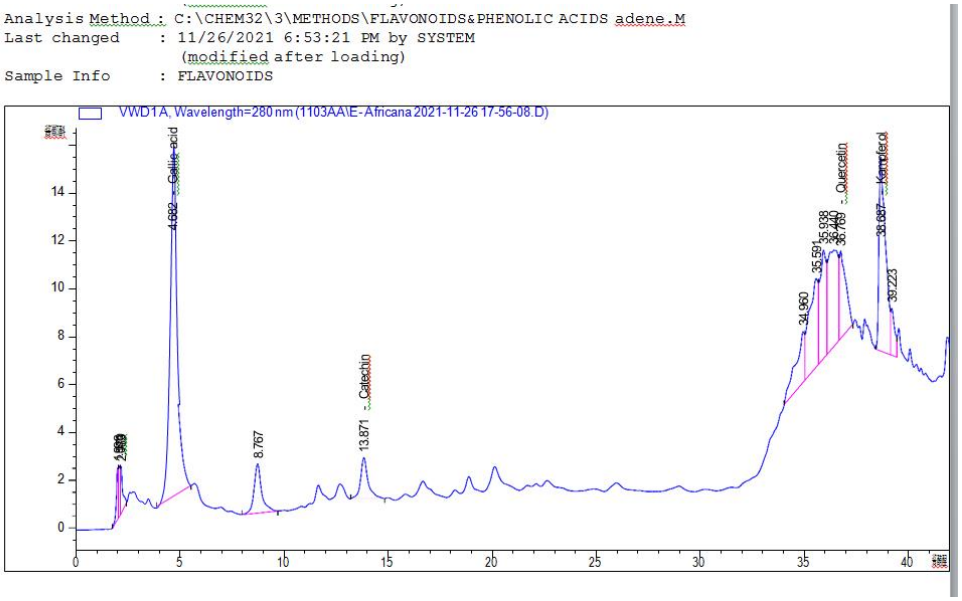


Figure 3.1: HPLC chromatogram of *Entada africana* showing various peaks of flavonoid rich compounds at a wavelength of 280 nm

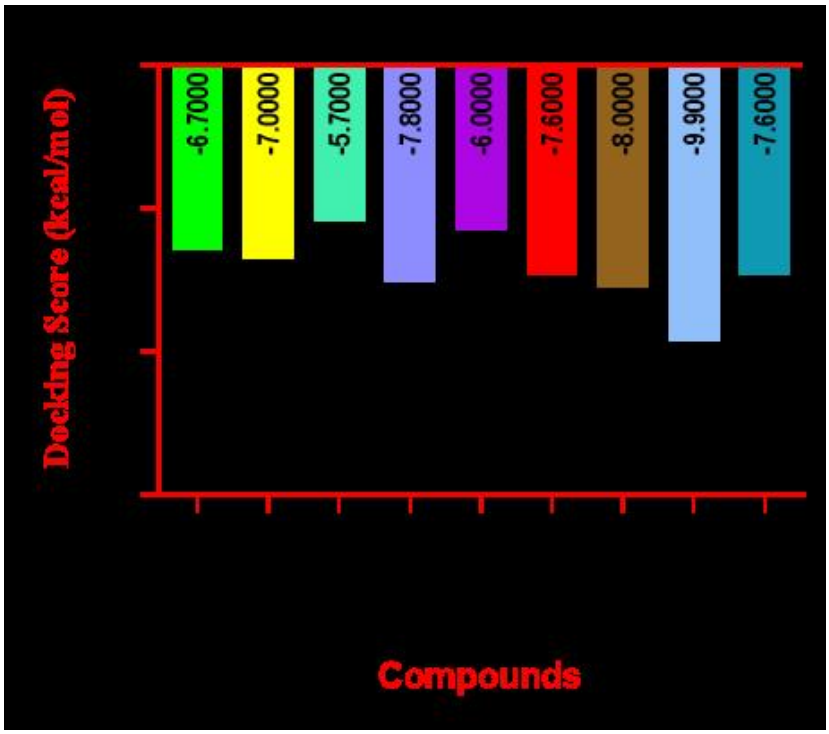


Figure 3.2: Binding affinities of the active compounds from *Entada africana* against heme polymerase viz: 2719 - Chloroquine; 323–Coumaric acid; 370 – Gallic acid; 9064 - Catechin; 445858–Ferulic acid; 5280343 - Quercetin; 5280443 - Apigenin; 5280805–Rutin; 5280863 – Kaemferol

Table 3.1 provides information about the number of hydrogen bonds formed between each of the docked pocket complexes and specific amino acids residues in the protein target. Hydrogen bond analysis reveals that rutin forms two hydrogen bonds with Ser 76 and Leu 74, while apigenin forms one hydrogen bond with Arg 27. Rutin (-9.9 kcal/mol) has a high binding energy in comparison to the standard drug, chloroquine (-6.7 kcal/mol) (Figure 4.7) which form no hydrogen bond with the amino acid residues (Table 4.2) due to the presence of the overall binding affinity. Similarly, ferulic acid (Asp 77, Ile 73 and Ala 29) and kaemferol (Arg

40, Arg 27 and Leu 74) form three hydrogen bonds. On the other hand, compounds like coumaric acid (Ala 291 and Arg 27), gallic acid (Ala 29 and Ser 76), catechin (Arg 40 and Arg 40) and quercetin (Ser 76 and Ser 76) form two hydrogen bonds with the amino acid residues.

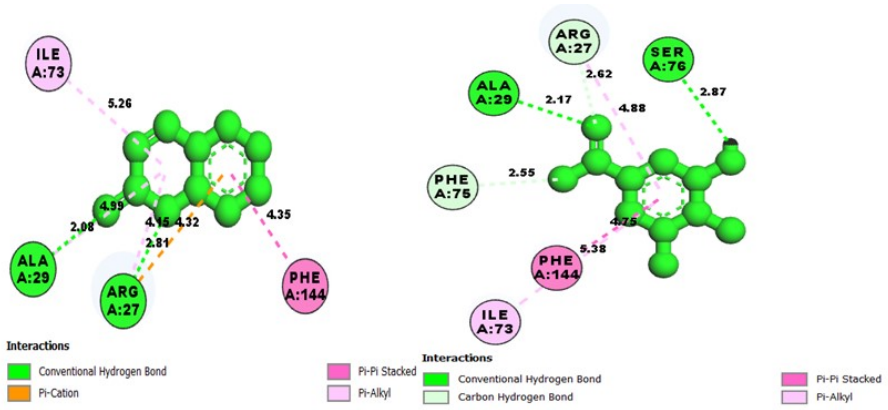
Chloroquine's inability to form a hydrogen bond may depend on the accuracy of the simulation parameters, force fields, and computational methods used. If these parameters are not well-suited for chloroquine or if there are limitations in the simulation conditions, the results may not accurately reflect the molecule's behavior.

Table 3.1: Hydrogen bond interaction of compounds with heme polymerase

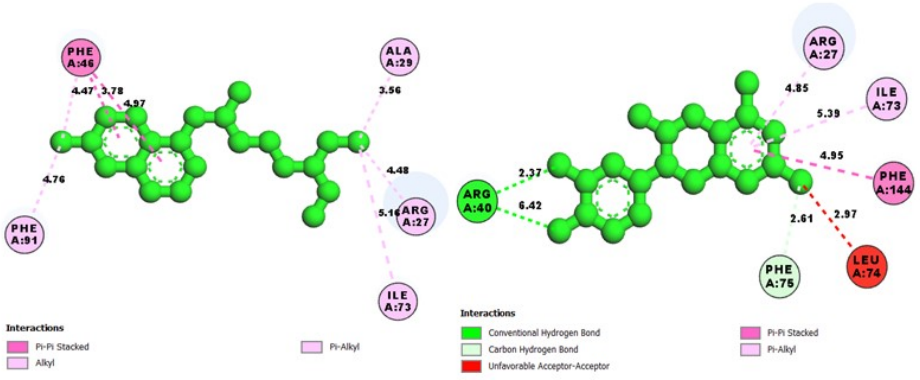
Compounds	Compound Name	No of H-bond	Interacting residues (distance Å)
2719	Chloroquine	Nil	Nil
323	Coumaric acid	2	ALA 291 (2.08Å); ARG 27 (2.81 Å)
370	Gallic acid	2	ALA 29 (2.17Å); SER 76 (2.87 Å)
9064	Catechin	2	ARG 40 (6.42 Å); ARG 40 (2.37Å)
445858	Ferulic acid	3	ASP 77 (1.96 Å) ILE 73 (2.91) ALA 29 (2.96)
5280343	Quercetin	2	SER 76 (2.85 Å); SER 76 (2.81 Å)
5280443	Apigenin	1	ARG 27 (2.43 Å)
5280805	Rutin	2	SER 76 (2.92 Å); LEU 74 (2.77 Å)
5280863	Kaemferol	3	ARG 40 (2.96 Å); ARG 27 (2.85 Å); LEU 74 (2.62 Å)

3D Interaction

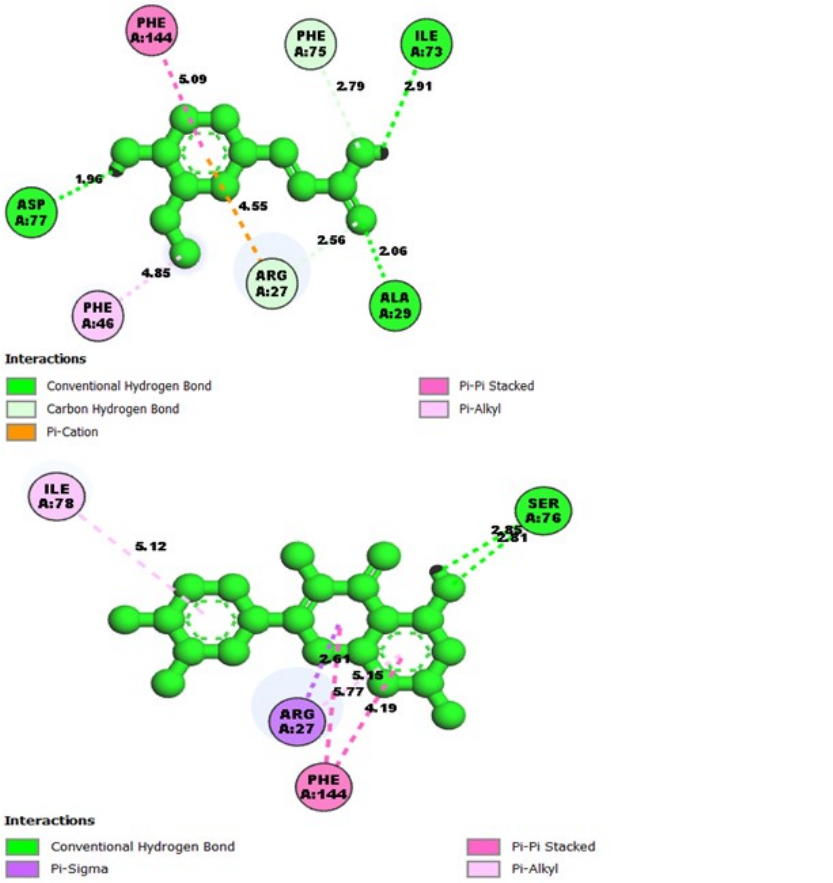
The interactions of compounds with the highest binding affinity with the active site amino acids of the targets, Coumaric acid (323), Gallic acid (370), Chloroquine (2719), Catechin (9064), Ferulic acid (445858), Quercetin (5280343), Apigenin (5280443), Rutin (5280805), and Kaemferol (5280863) diagrams were analyzed to identify the specific ligand binding site to heme polymerase (Figure 3.3)



323 370



2719 9064



445858 5280343

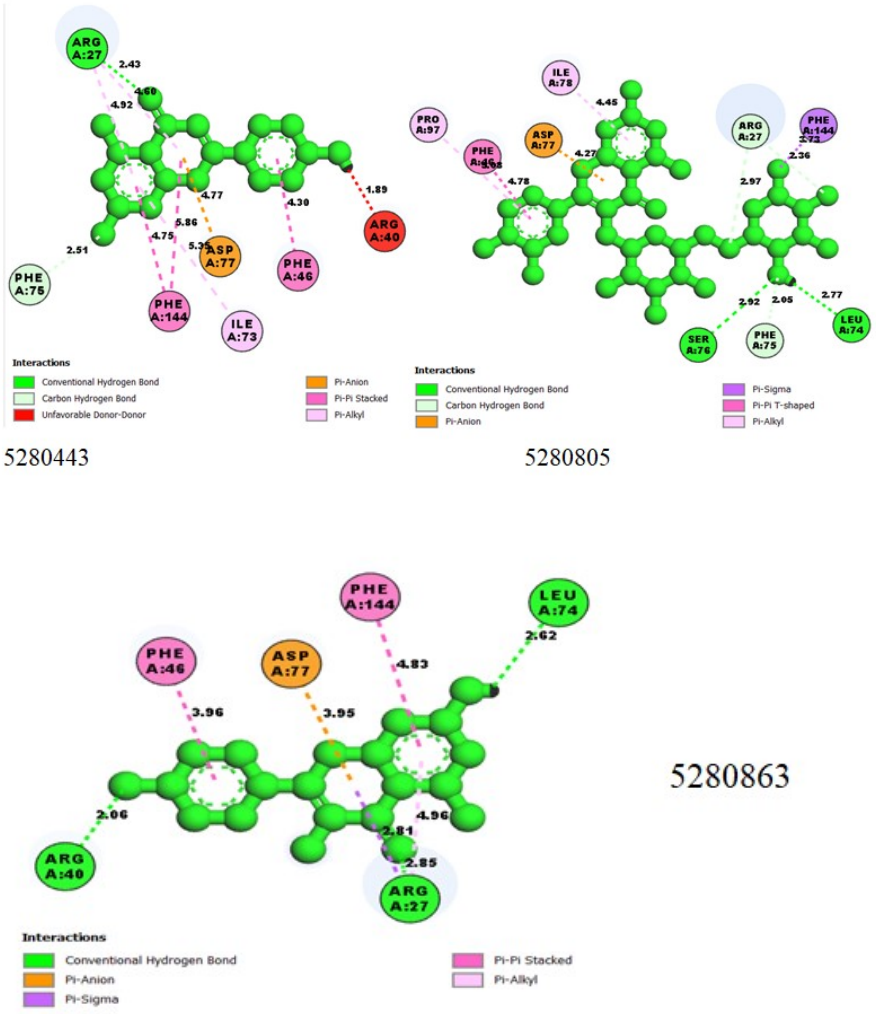


Figure 3.3: 3D representation of compounds-heme polymerase interactions viz: 323–Coumaric acid; 370 – Gallic acid; 2719 - Chloroquine; 9064 - Catechin; 445858–Ferulic acid; 5280343 - Quercetin; 5280443 - Apigenin; 5280805–Rutin; 5280863 – Kaemferol

Compounds Fitness Score via Pharmacophore Model

Fitness scores are used in quantitative structure-activity relationship studies. These scores evaluate how well a computational model predicts the biological activity of chemical compounds based on their structural features. High fitness scores indicate a better correlation between predicted and observed activities.

The result of the screening indicates that three compounds from MBEEA; quercetin, apigenin, and kaempferol obtained higher fitness scores (0.983) compared to other ligands (coumaric acid, gallic acid, catechin, ferulic acid and rutin) and the reference ligand, chloroquine (0.870) as shown in Table 4.3.

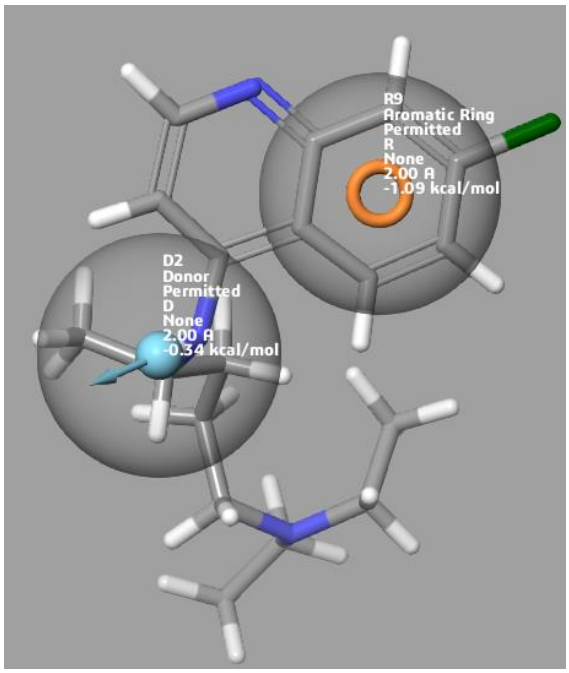


Figure 3.4: Pharmacophore hypothesis of the reference ligand (chloroquine) and the target (heme polymerase), hydrogen bond donor (D2; blue) and aromatic ring (R9; orange)

Table 3.2: Showing the compounds fitness score via pharmacophore model

Compound ID	Compound Name	Fitness Score
2719	Chloroquine	0.870
370	Gallic acid	0.459
5280343	Quercetin	0.983
5280443	Apigenin	0.983
5280863	Kaemferol	0.983
9064	Catechin	0.821
445858	Ferulic acid	0.459

Table 3.3: Predicted pIC_{50} for the compounds via QSAR model

Compound ID	Compound Name	pIC50 (μM)
2719	Chloroquine	5.245
323	Coumaric acid	4.844
370	Gallic acid	5.041
5280343	Quercetin	4.787
5280443	Apigenin	4.805
5280863	Kaemferol	4.805
9064	Catechin	4.944
4458585280805	Ferulic acid Rutin	4.8634.844

AutoQSAR modeling and pIC_{50} Prediction of compounds

Table 3.4: Parameters of the predicted model

Model code	S.D	R^2	RMSE	Q^2
kpls_radial_14	0.5483	0.5502	0.5521	0.4285

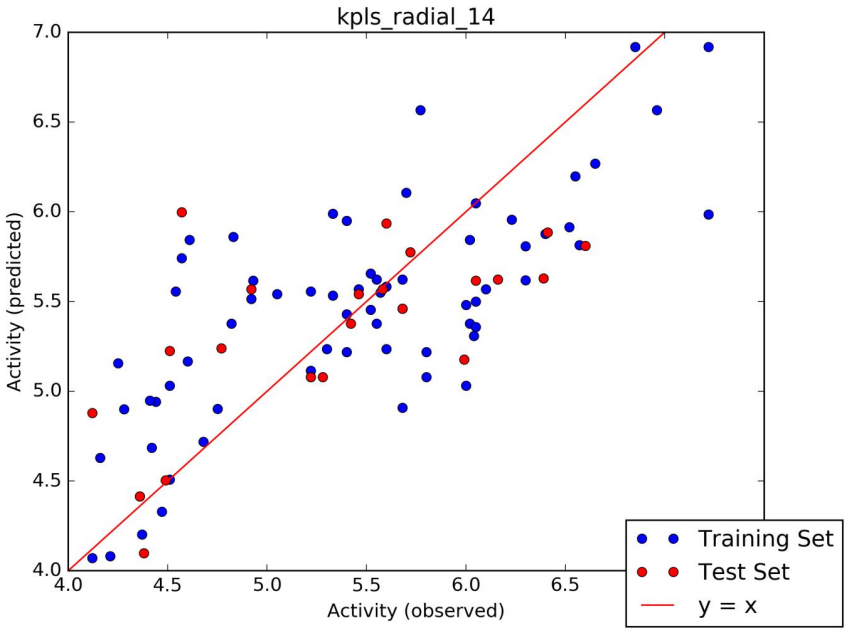


Figure 3.5: Scatter plot of pIC_{50} observed vs pIC_{50} predicted of QSAR model

Table 3.5: Predicted pIC_{50} for the compounds via QSAR model

Compound ID	Compound Name	pIC50 (μM)
2719	Chloroquine	5.245
323	Coumaric acid	4.844
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5280343	Quercetin	4.787
5280443	Apigenin	4.805
5280863	Kaemferol	4.805
9064	Catechin	4.944
4458585280805	Ferulic acid Rutin	4.8634.844

Pharmacokinetic Profile Prediction of Lead Compounds/ADMET Profiling

From Table 3.6, the result showed that the hit ligands are within the recommended range for blood/brain partition coefficient (-3.0 to 1.2) with rutin (-4.199) as an exception. A blood/brain partition coefficient value outside the recommended range, especially lower than -3.0, may suggest that rutin has lower permeability across the blood-brain barrier. This could be due to its molecular structure, charge, or other physicochemical properties. The results obtained revealed that gallic acid, ferulic acid, coumaric acid, have good $QPlog^{HERG}$ values ranging from (-1.396-3.845). Kaemferol, apigenin, quercetin, and chloroquine have poor $QPlog^{HERG}$ values and could block or inhibit hERG channels which could potentially lead to cardiac safety concerns. Only chloroquine and coumaric acid showed great calcium carbonate (Caco-2) cell permeability of 1525.824 nm/s and 2053.807 nm/s respectively observed to be greater than the reference value of 25 nm/s. Apigenin (-0.043), kaemferol (-0.201) and quercetin (-0.354) have a better

$QPlog^{khsa}$ values than the reference ligand, chloroquine (0.47). Gallic acid (4.348 nm/sec) and rutin (0.259 nm/sec) were observed with low QPP^{MDCK} values.

Table 3.7 showed the lead compounds are subjected to a structure-based pharmacokinetic screening using Admetlab elucidated pharmacokinetic behaviors of lead based on established pharmacokinetic descriptors such as molecular weight (Mol. Wt), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), topological surface area (TPSA), and Lipinski's rule violation (LRV).

Table 3.6: Pharmacokinetic prediction of hit molecules

Entry Name	$QPlog^{HERG}$	QPP^{Caco}	$QPlog^{BB}$	QPP^{MDCK}	$QPlog^{khsa}$
Chloroquine	-5.535	1525.824	0.45	2128.525	0.47
Coumaric acid	-3.845	2053.807	0.013	1076.936	-0.559
Gallic acid	-1.396	10.027	-1.659	4.348	-0.987
Quercetin	-5.035	20	-2.352	7.21	-0.354
Apigenin	-5.125	124.496	-1.411	52.038	-0.043
Kaemferol	-5.14	55.32	-1.843	21.655	-0.201
Catechin	-4.813	51.696	-1.91	20.125	-0.43
Ferulic acid	-2.06	77.348	-1.062	39.567	-0.624
Rutin	-4.597	0.921	-4.199	0.259	-1.195

Reference values: $Qplog^{HERG}$ IC_{50} value for blockage of HERG K^+channels (below –5); $QPlog^{BB}$ = -3.0 to 1.2; QPP^{Caco} =< 25 poor, > 500 great; $QPlog^{Khsa}$; binding to human serum albumin (-1.5 to +1.5), QPPMDCK: Apparent Madin-Darby canine kidney cell permeability in nm/sec.

Table 3.7: Drug likeness prediction of bioactive compounds

Entry Name	Mol. Wt	HBD	HBA	PSA	LRV
Chloroquine	319.876	1.000	4.000	24.094	1
Coumaric acid	146.145	0.000	2.500	40.767	0
Gallic acid	170.121	4.000	4.250	114.846	0
Quercetin	302.240	4.000	5.250	141.943	0
Apigenin	270.241	2.000	3.750	98.950	0
Kaemferol	286.240	3.000	4.500	120.544	0
Catechin	290.272	5.000	5.450	116.168	0
Ferulic acid	194.187	2.000	3.500	81.247	0
Rutin	610.524	9.000	20.550	270.703	3

Molecular weight (Mol. Wt), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), topological surface area (TPSA), Lipinski's rule violation (LRV).

Discussion

Molecular docking remains an important and established computational structural based virtual screening method employed in drug discovery and design. It predicts potential drug targets and molecular ligand-target interactions at the atomic level [8]. In this study, the molecular docking protocol was validated by preparation of the ligand, which is docked into the active site of the mod-

elled target (heme polymerase). Binding affinity plays a crucial role in drug discovery and development. Drug candidates are often designed or selected based on their high affinity for a target protein, such as an enzyme or receptor involved in a disease pathway [9]. High-affinity binding can lead to therapeutic efficacy. In Figure 3.2, the binding affinities of the compounds of MBEEA were compared with that of a standard antimalarial drug, chloroquine. Five of the assessed compounds bind better than the standard (chloroquine) at the active site of the protein, heme polymerase. These compounds are rutin (-9.9 kcal/mol), apigenin (-8.0 kcal/-mol), catechin (-7.8 kcal/mol), quercetin, and kaempferol (-7.6 kcal/mol) as compared to chloroquine (-6.7 kcal/mol). The improved binding affinities of these MBEEA compounds suggest that they have the potential to be more effective in inhibiting the activity of heme polymerase, a critical enzyme in the malaria parasite's life cycle. Malaria parasites require heme polymerase for heme detoxification, and inhibiting this enzyme can be a promising approach in antimalarial drug development [6]. To this end, the interactions diagrams were analyzed to identify the specific molecular interactions between the top-scoring compounds and the binding site of heme polymerase (Figure 4.8). The target, heme polymerase, is fast becoming an integral target in the fight against *Plasmodium*-borne diseases. It has been reported that the biosynthesis of malarial pigment (hemozoin) is catalyzed by heme polymerase [4]. The compounds of MBEEA showed multiple hydrogen bonding with the target, interactions likely to be one of the reasons the compounds bound better. Hydrogen bonding can influence the dynamics of protein-ligand interactions. They can affect the rate at which a drug associates and dissociates from its target, influencing the duration of the drug's pharmacological effect [22]. Table 3.1 provides information about the number of hydrogen bonds formed between each of the docked pocket complexes and specific amino acid residues in the protein target. Hydrogen bond analysis reveals that rutin forms two hydrogen bonds with SER 76 and LEU 74, while apigenin forms one hydrogen bond with ARG 27. Chloroquine forms no hydrogen bond with the amino acid residues. Similarly, ferulic acid (ASP 77, ILE 73 and ALA 29) and kaemferol (ARG 40, ARG 27 and LEU 74) form three hydrogen bonds. On the other hand, compounds like coumaric acid (ALA 291 and ARG 27), gallic acid (ALA 29 and SER 76), catechin (ARG 40 and ARG 40) and quercetin (SER 76 and SER 76) form two hydrogen bonds with the amino acid residues. Pi-pi stacking, pi-cation, pi-alkyl, pi-sigma, pi-anion, carbon hydrogen bond are a few examples of interactions that further stabilized the ligands at the ligand binding domain of heme polymerase [3]. Evidently, the antimalarial potency of chloroquine has a lot to do with its structure. The pharmacophore model is a computational representation of the essential features and constraints that a molecule needs to possess in order to interact with a specific biological target, such as a receptor or enzyme. These features include things like hydrogen bond donors/acceptors, hydrophobic regions, or specific functional groups, the pharmacophore model is constructed based on the known characteristics of the target and the expected binding interactions. The pharmacophore assessment was employed to determine which chemical interactions were key in the potency of chloroquine. Hydrogen bond donor and aromatic ring were two features identified by the pharmacophore screening as shown in Figure 3.4. The identification of the hydrogen donor feature in the pharmacophore assessment suggests that chloroquine likely forms hydrogen bonds with its target, heme polymerase. Hydrogen bonds are crucial for molecular recognition and binding between a drug and its target [13]. The presence of an aromatic ring in the pharmacophore suggests that chloroquine might be involved in pi-pi stacking interactions or hydrophobic interactions with its target [26]. In drug discovery and development, there are often large libraries of compounds to choose from. The fitness score helps prioritize and rank these compounds based on their potential to be effective drug candidates [23]. The fitness score is a numerical value that quantifies how well a given compound aligns with the features and constraints of the pharmacophore model, compounds with higher fitness scores are more likely to be selected for further testing and development. It is computed through a computational algorithm that assesses how closely the properties of a compound match the requirements of the model. Higher fitness scores indicate a better alignment, suggesting that the compound is more likely to bind effectively to the target [1]. The result of the screening indicates that three compounds from MBEEA—quercetin, apigenin, and kaempferol—obtained higher fitness scores compared to chloroquine. In computational terms, this means that these three natural compounds from the plant align very well with the features and constraints represented in the pharmacophore model, suggesting that they have a high potential to interact effectively with the target as seen in Table 3.2 Structure-activity relationship (SAR) is a critical aspect of drug development and computational chemistry, it involves studying the studying how the structural features of a compound (its chemical structure) relate to its biological activity [28].

The autoQSAR was used to assess the structure-activity relationship between the compounds of MBEEA and target; the result of

such assessment was summarized in Table 3.4. The negative logarithm of half maximal inhibitory concentration (pIC_{50}) of the ligands were also predicted (Table 3.5). pIC_{50} is a pharmacological term used to express the potency or concentration of a substance, typically a drug or compound, in inhibiting or modulating a specific biological activity. The lower the pIC_{50} value, the more potent the compound, as it indicates a lower concentration is required to achieve a half-maximal inhibitory effect. In the result shown in Table 4.6, the MBEEA compounds (gallic acid, ferullic acid, catechin, p-coumaric acid, rutin, apigenin, quercetin, and kaemferol) have pIC_{50} values comparable to chloroquine. In computational terms, the MBEEA compounds have pIC_{50} values that are in the same range or slightly lower than chloroquine, suggesting that they are similarly or potentially more potent than chloroquine, a known drug. Potential therapeutic agents are unable to enter clinical trials because of undesirable absorption, distribution, metabolism and excretion (ADMET) evaluations (5). Table 3.6 presents a comprehensive overview of pharmacokinetic predictions for a range of molecules, including chloroquine, coumaric acid, gallic acid, quercetin, apigenin, kaempferol, catechin, ferulic acid, and rutin. Each molecule's pharmacokinetic profile is evaluated based on various parameters: $QPlog^{HERG}$, QPP^{Caco}, $QPlog^{BB}$, QPP^{MDCK}, and $QPlog^{khsa}$, offering insights into their potential behavior within the human body.

$QPlog^{HERG}$, assesses the risk of human ether-a-go-go-related gene (hERG) inhibition. It was seen that chloroquine, Quercetin, Apigenin and Kaemferol exhibit values below -5, indicating a potential concern for hERG inhibition. This finding is crucial as hERG inhibition is associated with adverse cardiovascular effects. QPP^{Caco}, is a measure of Caco-2 cell permeability, chloroquine demonstrates high values, suggesting good absorption. Similarly, compounds, including coumaric acid and ferulic acid, also exhibit favourable values, indicating efficient absorption across the intestinal membrane. The $QPlog^{BB}$ parameter evaluates blood-brain barrier penetration. All the compounds except rutin fall within the optimal range, indicating their potential to penetrate the blood--brain barrier adequately. This is a significant consideration, especially for compounds intended to target the central nervous system. QPP^{MDCK} values, associated with permeability through Madin-Darby Canine Kidney cells. Chloroquine, coumaric acid, apigenin, and ferulic acid are generally predicted to have high values, leaving Chloroquine and Coumaric acid with the highest values. This suggests good permeability, emphasizing the potential for these molecules to traverse biological membranes effectively. Additionally, $QPlog^{khsa}$ provides insights into binding to human serum albumin. All the compounds exhibit values close to zero (0) or slightly negative, indicating a lower likelihood of strong binding to albumin. This is relevant as it influences the distribution and availability of the compounds in the bloodstream. A higher binding affinity is not enough to make a compound a successful drug candidate; as a result, the drug-likeness profiles of bioactive compounds of MBEEA were also assessed (Table 3.7). Hence, evaluating of ADME status of small molecules is important in drug discovery. *In silico* approach is the cheapest and fastest strategy for screening large compounds for safety. Drug likeness is a concept required to qualitatively predict the possibility of molecules becoming an oral drug, describing the molecular importance of the drug's pharmacokinetic properties in the body. The popular Lipinski's rule of five (ROV) is related to this concept, according to Lipinski's ROV, an orally active drug should have the following properties: donor hydrogen bond less than 5 (HBD <5), acceptor hydrogen bond less than 10 (HBA <10), molecular weight less than 500 Da (MW < 500 Da), and octanol-water partition coefficient less than 5 (LogP<5). The rule states that, a drug-like molecule must not violate more than one of the rules of five (10). The results shown on Table 3.7 revealed that, except for rutin, which had more than five hydrogen bond donors, molecular weight greater than 500Da and hydrogen bond acceptor greater than 10, thereby violating three Lipinski's rule, other hit compounds including the reference drug, chloroquine obeyed all the Lipinski's rule of five. This suggests that, all the hit compounds can be predicted as good therapeutic candidate. Overall, the top compounds of MBEEA not only bound heme polymerase effectively but also demonstrated excellent pharmacokinetic and pharmacodynamic signatures, necessitating the need to further subject these compounds to *in vivo* assay in the total elucidation of the seroclearance potentials in the long fight against *Plasmodium* -borne infections.

Conclusion

The results of this study highlight the intriguing possibility of substances obtained from MBEEA as formidable contenders against *Plasmodium*-borne illnesses, including malaria. It was shown by molecular docking analyses that a number of MBEEA compounds

have better binding affinities to the target protein, heme polymerase, than the common antimalarial medication, chloroquine. Notably, rutin, apigenin, catechin, quercetin, and kaempferol showed significantly higher binding affinities, suggesting that they can block heme polymerase, an enzyme that is vital to malaria parasite viability. Subsequent analyses of the molecular interactions between the heme polymerase binding site and the highest-scoring compounds revealed several hydrogen bonds as well as other stabilizing interactions. These interactions are a major factor in the compounds' increased binding affinities, which may indicate how they can work as antimalarial drugs. Hydrogen bond donor and aromatic ring characteristics were found to be critical for the critical chemical interactions that contribute to chloroquine's potency, as revealed by pharmacophore modelling. Contrary to chloroquine, molecules from MBEEA showed better fitness ratings, suggesting that they may be more effective at interacting with the target and that they had great agreement with the pharmacophore model. Furthermore, molecules from MBEEA have similar or even greater potencies than chloroquine, according to structure-activity relationship (SAR) studies and the prediction of pharmacological characteristics like pIC_{50} values. Additionally, as determined by Lipinski's rule of five, their drug-likeness profiles were favourable. Additionally, the MBEEA compounds' favourable pharmacokinetic profiles were revealed by the assessment of ADME (absorption, distribution, metabolism, and excretion) features, indicating their potential applicability for further development as oral medicinal agents. Although rutin deviated from several of Lipinski's parameters, most of the hit compounds, such as the reference medication chloroquine, complied with them, suggesting that they could be effective therapeutic candidates.

This study employed a combination of molecular docking, pharmacophore modeling, and QSAR analysis, providing a comprehensive understanding of the binding interactions and structural requirements for the heme polymerase inhibition ensuring relevance and accuracy of the results.

Limitations of the Study

This study relied heavily on computational models with limited data size, potentially overlooking complex biological interactions which may not always accurately represent real-world scenerios.

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