Journal of Community Health Provision
Vol. 6, Issue 2, 2026
Page 596-611
DOI: https://doi.org/10.55885/jchp.v6i2.1022

Antiplasmodial Activity and Interactions of *Persea americana* Seed Flavonoids Against Plasmepsin II

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Abstract. *Malaria caused by Plasmodium falciparum remains a major global health burden, and the increasing resistance to conventional antimalarial drugs urgently demands the discovery of novel bioactive compounds from natural sources. This study aimed to evaluate the antiplasmodial potential of methanol extract from Persea americana seeds and to identify its active phytochemical constituents through an integrated experimental and computational approach. The research design combined phytochemical profiling, in vitro antiplasmodial bioassay, and molecular docking simulation, contributing a mechanistic correlation between phytochemical composition and target-specific inhibitory activity-an aspect that remains insufficiently explored for this plant species. Phytochemical characterization was performed using high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS). The in vitro antiplasmodial activity was evaluated against the P. falciparum strain 3D7 using a four-parameter logistic model, and molecular docking was performed against Plasmepsin II (PDB ID: 1LF3) using AutoDock Vina. HPLC analysis revealed the presence of seven flavonoids, of which rutin was the major compound (84.15%), followed by epicatechin (10.59%). GC–MS analysis detected volatile constituents, including avocadene acetate, avocadenofuran, linoleic acid, and palmitic acid. The extract showed high antiplasmodial activity with an IC_{50} of 3.41 µg/mL against P. falciparum 3D7, which was within the WHO threshold for highly active plant extracts. Molecular docking revealed that kaempferol exhibited the strongest binding affinity toward Plasmepsin II (−8.10 kcal/mol), closely approaching chloroquine (−8.44 kcal/mol), and directly engaged the catalytic residue ASP214. These results demonstrate that P. americana seeds are a promising source of antiplasmodial compounds, and that structural compatibility with the target enzyme is a stronger determinant of binding affinity than compound abundance, positioning kaempferol as a lead candidate for further enzymatic and in vivo investigation.*

Keywords: *Antiplasmodial Activity, Flavonoids, Persea Americana Seed, Plasmepsin II, Plasmodium Falciparum*

Received: May 20, 2026 Received in Revised: July 6, 2026 Accepted: August 25, 2026

INTRODUCTION

Malaria remains a major global public health challenge, with an estimated 282 million cases and 610,000 deaths recorded worldwide in 2024, mainly in tropical high-transmission regions. *Plasmodium falciparum*, the most lethal species, is responsible for most severe malaria cases and deaths, especially in sub-Saharan Africa (Li et al., 2024). The emergence and rapid spred of drug-resistant parasite strains worldwide have greatly reduced the effectiveness of available

antimalarial drugs, necessitating the urgent need to identify and develop novel treatment candidates (Uwimana et al., 2020; Siqueira-Neto et al., 2023; Shibeshi et al., 2020; Duffey et al., 2024; Sanyaolu et al., 2025). Natural products are a rich source for antimalarial drug discovery, with the isolation of quinine from *Cinchona* spp. and artemisinin from *Artemisia annua*, which remain important components of malaria treatment (Newman & Cragg, 2020; Pohlit et al., 2013; Tu, 2011).

Among plant-derived compounds, flavonoids have attracted considerable attention due to their structural diversity and broad biological activities. Several studies have demonstrated antiplasmodial effects of flavonoids through multiple mechanisms, including disruption of parasite metabolism and redox homeostasis (Tajuddeen & Van, 2019; Kumar, 2026).

Persea americana (avocado) is a widely cultivated crop in tropical regions and its seeds that often discarded as agro-industrial waste. These seeds are rich in several bioactive compounds, especially flavonoids and phenolic compounds, which have demonstrated antioxidant, antimicrobial, and anti-inflammatory activities (Dabas et al., 2013; Fidelis t al., 2019; Dalli et al., 2021; Quintana et al., 2021). Previous studies have reported that ethanol and chloroform extracts of *P. americana* seeds possess significant antiprotozoal activity, with phenolic compounds such as catechin and epicatechin being identified as active constituents (Jiménez-Arellanes et al., 2013; Siol & Sadowska, 2023; Uzor et al., 2021). However, there is limited research specifically investigating the antiplasmodial potential of *P. americana* seed extract against defined molecular targets, supported by comprehensive phytochemical profiling.

In vitro assays provide a direct, quantitative method for assessing antiplasmodial activity by measuring the inhibitory effects of test compounds on the growth of *P. falciparum* under controlled laboratory conditions (Bouzón-Arnáiz et al., 2022; Walz et al., 2023; Lucantoni et al., 2016; Vaidya et al., 2014; Fokou et al., 2025). *In silico* molecular docking allows prediction of ligand–protein interactions and offers mechanistic insight at the molecular level (Ferreira et al., 2015). Plasmepsin II (PDB ID: 1LF3) is a key molecular target, an aspartic protease catalyzing the degradation of hemoglobin in the digestive vacuole of *P. falciparum*.

This enzyme catalyzes the initial cleavage of host hemoglobin, providing essential amino acids for parasite growth; its inhibition interferes with this process, leading to parasite death. The mature structure of plasmepsin II contains two catalytic aspartyl residues, Asp34 and Asp214, which are crucial for its proteolytic activity and thus ideal targets for structure-based inhibitor design (Cheuka et al., 2020; Ji et al., 2022; Jin et al., 2014). Recent computational studies have shown that a number of flavonoids are more potent binders with higher binding energies against plasmepsin II, thus supporting the relevance of this target in flavonoid-based drug discovery (Olatunde et al., 2025; Syed et al., 2025).

Therefore, the present work aims to study the antiplasmodial activity of *Persea americana* seed extract by combining phytochemical profiling using HPLC and GC–MS, *in vitro* evaluation, and molecular docking against plasmepsin II. This approach is designed to establish a mechanistic correlation between dominant phytochemical constituents and their interactions with key catalytic residues, thereby identifying potential compounds responsible for enzyme inhibition and parasite growth suppression.

METHODS

The study, based on an integrated experimental and computational design, was conducted to evaluate the antiplasmodial potential of *Persea americana* seed extract. This work was performed in three stages (1) extensive phytochemical characterization of the seed extract using HPLC and GC–MS to classify and characterize the biologically active compounds present, (2) In vitro assay for antiplasmodial activity against *P. falciparum* strain 3D7, and (3) In silico molecular docking of major flavonoid characters from HPLC profiling with plasmepsin II—(PDB ID: 1LF3) as the primary molecular target. This multi-level strategy was aimed to directly and

mechanistically correlate extract phytochemical composition with the biological activity observed.

Plant Material and Extraction

Avocado seeds were obtained from ripe fruits purchased from a traditional market in Pekanbaru, Riau Province, Indonesia. The cultivar of the fruits could not be determined because the samples were sourced from a local market. Fruits were selected at commercial ripeness based on peel color and firmness and were processed immediately after purchase. The seeds were thoroughly washed to remove residual pulp, cut into thin slices, and air-dried at room temperature in a shaded area until sufficiently dry. The dried seeds were then ground into a fine powder and stored in a tightly closed dark container until extraction. Extraction was performed by maceration using methanol (99%, technical grade) at a ratio of 1:10 (w/v). The powdered material was soaked for 72 h at room temperature and subjected to ultrasonic bath treatment for 30 min to enhance extraction efficiency (Fadhli et al., 2018). The mixture was filtered through Whatman No. 1 filter paper, and the residue was remacerated twice with fresh solvent under identical conditions. All filtrates were combined and concentrated using a rotary evaporator at a temperature below 45°C. Further solvent removal was performed using a low-temperature water bath to obtain a concentrated reddish-brown extract. The extraction process yielded 180 g of concentrated extract from 2000 g of dried seed powder, corresponding to an extraction yield of 9% (w/w). The final extract was stored in dark vials at 4°C until further phytochemical characterization and antiplasmodial evaluation.

Phytochemical Analysis Using HPLC and GC–MS

Phytochemical profiling of main components in *P. americana* seed extract was performed by means of high-performance liquid chromatography (HPLC) and gas-phase chromatography–mass spectrometry (GC–MS). HPLC was carried out on Thermo Scientific Vanquish system equipped a diode array detector (DAD). The experimental protocol included a 5 µL sample solution injection and a 30 min chromatographic separation. The detector was used to monitoring of analytes at 210, 254, 280 and 320 nm. Data were analyzed as method chromatograms on Chromeleon software (3D quantitative flavonoid method, chromatographic data via Chromeleon software). Analyses were executed in reversed-phase C18 column (250 mm × 4.6 mm, 5 µm). The gradient mobile phase was acetonitrile and 0.1% formic acid in water, starting 20% to 60% for 30 minutes. The method was carried out at a constant flow rate of 0.5 mL/min and isocratic column temperature of 25 °C, detection wavelengths: 210, 254, 280 and 360 nm. Identity of each compound was determined in reference to its retention time and spectral data, based on comparison with reference standards and literature values (Mizzi et al., 2020). Quantitative method validation parameters (linearity, limit of detection, limit of quantification, precision, and recovery) were not established for this HPLC method; the reported relative area percentages therefore reflect semi-quantitative compositional proportions within the chromatogram rather than absolute, validated concentrations, and this is acknowledged as a limitation of the present study. GC–MS analysis was carried out on a Thermo Scientific TRACE 1610 gas chromatograph coupled with an ISQ 7610 single quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), fitted with TriPlus RSH autosampler. Chromatographic separation was performed on a TG-WAXMS polar capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Thermo Fisher Scientific). Each sample solution was injected (1 µL) into the split mode with a ratio of 50:1; In this case, the injector temperature was maintained at 230 °C and septum purge flow of 5.0 mL/min. The mass spectrometer scan was performed with the following oven program: an initial hold at 50 °C for 2 min, followed by a ramp of 5 °C/min to 280 °C which was held for 2 min with a total time run of 49.89 min. Mass spectra were obtained in full-scan mode (m/z 20–550) with electron ionization (EI; 70 eV), transfer line temperature of 260 °C, and ion source temperature of 220 °C. Data acquisition and processing were performed using Chromeleon chromatography software (version 7.3.2). Compounds were identified by matching the mass spectra against the NIST library, retaining only constituents with a library match quality score of 700 or higher (out of 999) as reliably identified; compounds below this threshold were

excluded from Table 2. Identification was not further confirmed by retention index calculation or comparison with authentic reference standards, which is acknowledged as a limitation of the present study.

In Vitro Antiplasmodium Bioassay

Antiplasmodial activity was evaluated against *P. falciparum* strain 3D7, obtained from the Tropical Disease and Diagnostic Center, Universitas Airlangga, Surabaya, Indonesia. Parasite cultures were maintained as described in the modified Trager and Jensen method. Stock solution was prepared by dissolving 10 mg of extract in 1000 µL dimethyl sulfoxide (DMSO; Merck, ≥99.9%). The final DMSO concentration in all the assay wells was maintained at ≤0.1% (v/v), and a DMSO vehicle control at an equivalent concentration was included in all the assay plates to exclude any solvent related cytotoxic effects. Serial dilutions were prepared to give final test concentrations of 100, 50, 10, 1, 0.1, and 0.01 µg/mL. Parasites were synchronized to the ring stage and cultures were adjusted to approximately 1% parasitemia with 5% hematocrit. The assay was carried out in 96-well microplates using 2 µL test solution and 198 µL parasite suspension, in triplicate for each concentration. Plates were incubated at 37 °C for 48 h in a sealed chamber with 5% O_2, 5% CO_2, and 90% N_2. After incubation, thin blood smears were prepared, stained with 20% Giemsa solution and observed under a light microscope. Parasitemia was determined by counting infected erythrocytes per 1000 total erythrocytes. The experiment was independently repeated on three separate occasions (three biological replicates). Chloroquine diphosphate (Sigma-Aldrich) was used as the reference drug.

Data Analysis and Four Parameter Logistic Model

A dose–response analysis was evaluated to determine the relationship between extract concentration and the growth of *P. falciparum*. The percentage inhibition was determined by the measured parasitemia values after 48 h incubation compared with the negative control. The reliability of the data was guaranteed using mean values obtained from triplicate experiments. Percentage growth was used as response variable for the construction of a dose-response curve. The dose–response relationship was analyzed using a four-parameter logistic (4PL) nonlinear regression model to estimate the IC_{50} value, defined as the concentration required to inhibit 50% of parasite growth (Sebaugh, 2011). Nonlinear regression was performed by the least squares method, with concentration values converted to a base-10 logarithmic scale. Curve fitting was performed using the log (inhibitor) versus response model with variable slope implemented in GraphPad Prism version 10.6.1 (GraphPad Co. Ltd., San Diego, CA, USA). The model defines IC_{50} as the concentration producing a response halfway between the minimum and maximum asymptotes. The IC_{50} value derived from the fitted curve was used as the primary indicator of antiplasmodial activity.

In Silico Molecular Docking

Molecular docking simulation was performed to predict the binding interactions between selected bioactive flavonoids and plasmepsin II (PDB ID: 1LF3), where the three-dimensional structure of plasmepsin II was retrieved from RCSB Protein Data Bank (https://www.rcsb.org). Protein preparations were performed using BIOVIA Discovery Studio Visualizer v20. 1. 0. 19295 (BIOVIA, Dassault Systèmes), using water removal and co-crystallized ligands removal, then adding hydrogen atoms and appropriate protonation states so as not to disturb the structure when docking. In order to check the consistency of the docking protocol, a redocking procedure was implemented in which the native ligand was redocked into plasmepsin II active site. The docking method was validated by measuring the root mean square deviation (RMSD) between the predicted orientation of the pose and that of crystallographic one. Values of RMSD less than 2.0 Å means that the binding mode has been accurately recreated, while values from 2.0 to 3.0 Å are still considered acceptable because of flexibility and conformational variability (Ramírez & Caballero, 2018).

Ligand selection was based on phytochemical profiling and drug-likeness criteria. Flavonoid compounds identified by HPLC analysis were screened according to Lipinski's Rule of Five (Lipinski et al., 2001). Four compounds: kaempferol, rutin, myricetin, and quercetin, were selected for docking analysis. Troxerutin was omitted because of its unusual chromatographic behavior (zero detector height response) that means no resolution was observed to confidently assign a structure (Cheuka et al., 2020; Jin et al., 2014). Epicatechin, although being the second most abundant compound (10.59%), is a flavan-3-ol and does not have the 3-hydroxy group on the C-ring and the 4-keto group of the flavonol group, which are required for hydrogen bond formation with the catalytic aspartyl residues of Plasmepsin II (Cheuka et al., 2020; Jin et al., 2014). Daidzein, being an isoflavone, also lacks the structural features required for optimal interaction with the enzyme active site. Both compounds were therefore excluded from the primary docking analysis; however, supplementary docking of epicatechin is recommended in future studies to empirically verify this exclusion. The four selected ligands were retrieved from the PubChem database in SDF format and prepared using ChemDraw Professional 19.1.0.8 (PerkinElmer). Energy minimization was performed using the MMFF94 force field in Chem3D Professional to generate energetically stable three-dimensional conformations prior to docking.

The docking simulations were performed using Autodock Vina (through AutoDock Tools1.5.7) All protein and ligand structures were converted to PDBQT and Gasteiger charges were assigned. The region of interest (ROI) for each ligand was defined in terms of a docking grid box, which included the active pocket of plasmepsin II; specifically, catalytic residues Asp34 and Asp214 were included. The grid center was placed at (x = 15.785, y = 5.126, z = 26.424) with sizes of 60 × 60 × 60 grid points and spacing of 0.375 Å to cover the binding pocket well. Docking calculations were performed with an exhaustiveness parameter of 8 with the best binding poses being chosen based on lowest binding energy values. The resulting docking conformations were analyzed on the basis of binding affinity and interaction profiles. Protein–ligand interactions such as hydrogen bonding, π-interactions, and hydrophobic contacts, were visualized and analyzed using BIOVIA Discovery Studio Visualizer. Particular attention was given to interactions with key catalytic residues to elucidate potential inhibitory mechanisms. All computational procedures were carried out on an ASUS Zenbook 14 (UX3405MA) laptop equipped with an Intel Core Ultra 7 155H processor (3.80 GHz), 32 GB RAM, Intel Arc Graphics, and running on a 64-bit Windows 11 Home operating system.

RESULT AND DISCUSSION

HPLC Phytochemical Profiling

Table 1. HPLC Phytochemical Profiling of Avocado Seed Extract

No.	Compound	RT (min)	Area (mAU·min)	Height (mAU)	Rel. Area (%)	Rel. Height (%)
1	Epicatechin	9.11	6.35	57.82	10.59	12.68
2	Rutin	11.33	50.48	372.37	84.15	81.67
3	Troxerutin	11.88	0.53	0	0.89	0
4	Myricetin	13.58	0.61	4.80	1.03	1.05
5	Daidzein	15.18	0.70	6.68	1.17	1.47
6	Quercetin	15.69	0.76	9.18	1.27	2.01
7	Kaempferol	17.70	0.54	5.08	0.90	1.11
Total	—	—	59.99	455.94	100	100

HPLC analysis of the *P. americana* seed methanolic extract successfully identified seven flavonoid compounds eluting between 9.11 and 17.70 minutes, as illustrated in the chromatogram (Figure 1) and summarized in Table 1. Rutin emerged as the overwhelmingly dominant constituent, accounting for 84.15% of the total relative area at a retention time of 11.33 minutes, followed by epicatechin as the second major compound at 10.59% eluting earliest at 9.11 minutes. The remaining five compounds troxerutin (0.89%; RT 11.88 min), myricetin

(1.03%; RT 13.58 min), daidzein (1.17%; RT 15.18 min), quercetin (1.27%; RT 15.69 min), and kaempferol (0.90%; RT 17.70 min) were detected in minor but nonetheless meaningful quantities. Notably, troxerutin displayed a peak area of 0.53 mAU·min with a height of 0 mAU, which likely reflects a very broad, flat peak whose maximum intensity fell below the detector height threshold at the selected wavelength, rather than a complete absence of signal. The sequential elution pattern observed reflects the differing polarities among these compounds, whereby glycosylated flavonoids such as rutin eluted earlier than their aglycone counterparts — a chromatographic behavior attributable to the hydrophilic nature of the sugar moiety, which reduces compound retention on the non-polar stationary phase in RPHPLC systems (Hu et al., 2025; Ismail & Butnariu, 2024).

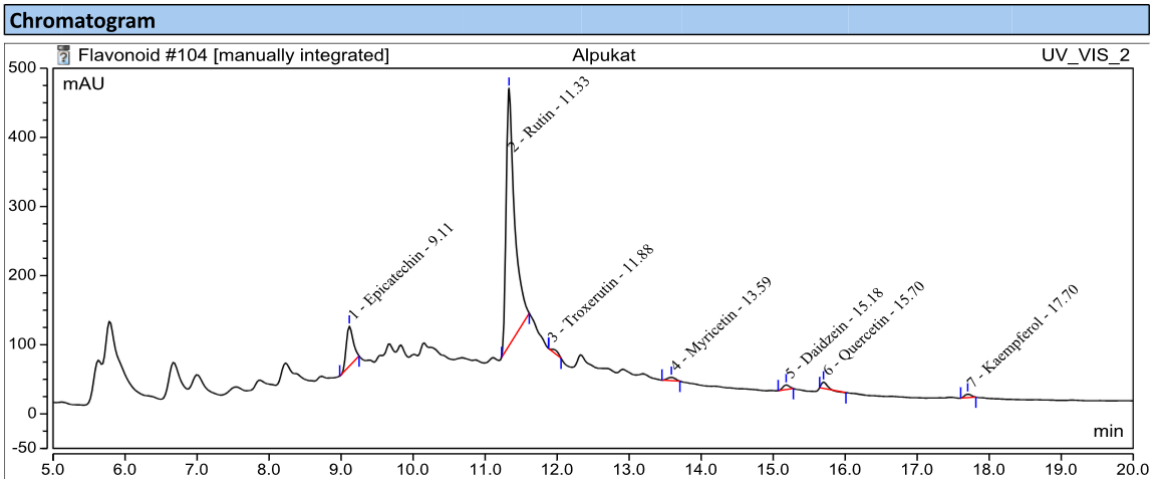


Figure 1. HPLC chromatogram of *Persea americana* seed methanolic extract showing seven identified flavonoid compounds at 280 nm. Peak identification: (1) Epicatechin, (2) Rutin, (3) Troxerutin, (4) Myricetin, (5) Daidzein, (6) Quercetin, (7) Kaempferol

The predominance of rutin, a glycosylated flavonoid, is largely due to the selective affinity of methanol for glycosylated flavonoids over their aglycone counterparts, which also explains the disproportionately low recovery of quercetin and kaempferol despite their known biological potency (Dabas et al., 2013). This extraction-driven bias raises a plausible hypothesis, rather than a confirmed relationship, that compounds present in the smallest quantities may nonetheless contribute meaningfully to pharmacological activity. Aglycone flavonoids like quercetin and kaempferol possess free hydroxyl groups on the B-ring and C-3 positions, structural features theoretically favorable for interaction with the catalytic residues of plasmepsin II, a key proteolytic enzyme involved in hemoglobin degradation in *Plasmodium* parasites (Tajuddeen & Van, 2019). On the other hand, the rutinose moiety of rutin, although abundant, introduces steric hindrance, likely limiting its molecular access to the enzyme active site (Slámová et al., 2018). This divergence where rutin dominates quantitatively yet may underperform in targeted molecular binding, while kaempferol and quercetin remain scarce yet structurally favorable for enzymatic inhibition is proposed here as a working hypothesis to be examined further through molecular docking analysis; confirming that kaempferol or quercetin are directly responsible for the observed antiplasmodial activity would require bioassay-guided fractionation or testing of the isolated compounds, which was beyond the scope of the present study (Penna-Coutinho et al., 2018).

GC–MS volatile and Semi-Volatile Phytochemical Composition

GC–MS analysis of *Persea americana* seed methanol extract identified 10 major volatile and semi-volatile constituents (relative area ≥4.00%) belonging to six chemical classes, with library quality match scores of 766–909 (Table 2). The total ion chromatogram resolved 22 peaks over a run time of 49.89 min, confirming the chemical diversity of this extract.

Table 2. Major GC–MS Constituents (relative area ≥4.00%) of *Persea americana* Seed Methanol Extract

No	Peak	RT (min)	Area (%)	Compound (Best Match)	CAS No.	Qual	Formula	MW	Class
1	1	27.30	11.90	Dihydroxyacetone	96-26-4	873	C3H6O3	90	Polyol
2	2	27.73	9.67	Avocadenofuran	25346-24-1	891	C17H28O	248	Furanoid terpenoid
3	7	31.33	4.73	16-Heptadecyn-4-one, 1,2-dihydroxy-	1379770-10-1	909	C17H30O3	282	Furanoid terpenoid
4	10	34.65	5.12	5-Hydroxymethylfurfural (5-HMF)	67-47-0	867	C6H6O3	126	Furan aldehyde
5	11	35.24	5.84	2-((8Z,11Z)-Heptadeca-8,11-dien-1-yl) furan	148675-93-8	864	C21H34O	302	Furanoid terpenoid
6	12	40.78	7.73	n-Hexadecanoic acid (palmitic acid)	57-10-3	826	C16H32O2	256	Fatty acid
7	18	44.81	8.17	9,12-Octadecadienoic acid (Z,Z)- (linoleic acid)	60-33-3	839	C18H32O2	280	Unsaturated fatty acid
8	19	45.73	4.55	Octaethylene glycol monododecyl ether	3055-98-9	766	C28H58O9	538	Glycol ether
9	21	48.13	15.46	Avocadene acetate	24607-09-8	797	C19H36O4	328	Terpenoid acetate
10	22	48.90	4.08	Octaethylene glycol monododecyl ether	3055-98-9	797	C28H58O9	538	Glycol ether

Notes: RT, retention time; Qual, quality match score (NIST/Wiley library); MW, molecular weight.

Avocadene acetate (peak 21; RT 48.13 min; 15.46%) was identified as the most abundant compound, followed by dihydroxyacetone (peak 1; RT 27.30 min; 11.90%), avocadenofuran (peak 2; RT 27.73 min; 9.67%), linoleic acid (peak 18; RT 44.81 min; 8.17%), and palmitic acid (peak 12; RT 40.78 min; 7.73%). The total peak area was approximately 22% of several furanoid terpenoids, including avocadenofuran (peak 2), 16-heptadecyn-4-one, 1,2-dihydroxy- (peak 7; RT 31.33 min; 4.73%), and 2-((8Z,11Z)-heptadeca-8,11-dien-1-yl) furan (peak 11; RT 35.24 min; 5.84%). These compounds are characteristic constituents of *Persea* species and have been previously linked to antimicrobial and antiprotozoal activities (Bangar et al., 2022; Miramontes-Corona et al., 2024). Linoleic acid (peak 18; RT 44.81 min; 8.17%) and palmitic acid (peak 12; RT 40.78 min; 7.73%) were the major fatty acid constituents. Linoleic acid has been reported to disrupt *Plasmodium* membrane integrity, as the parasite cannot synthesize polyunsaturated fatty acids de novo and depends on host-derived lipids for membrane remodeling (Amrodi et al., 2023). As the extract was tested as a whole, whether linoleic acid contributes to this mechanism within the present extract was not directly examined and remains a plausible contributing factor rather than a confirmed one. Palmitic acid has similarly been reported to inhibit *P. falciparum* enoyl-ACP reductase (PfENR/FabI) in the type II fatty acid synthase (FAS-II) pathway, a selective target absent in mammalian hosts (Bangar et al., 2022). As with linoleic acid, this effect was demonstrated for the isolated compound in previous work and was not directly tested for the present extract. 5-Hydroxymethylfurfural (5-HMF; peak 10; RT 34.65 min; 5.12%) was identified as a minor furan aldehyde constituent.

The GC–MS volatile profile complements the HPLC flavonoid data (Table 1), together supporting a multi-target antiplasmodial model in which polar flavonoids (rutin and epicatechin) and non-polar terpenoid-lipid fractions act in concert against distinct *P. falciparum* targets (Uzor,

2020; Uzor et al., 2021). Dihydroxyacetone (peak 1; RT 27.30 min; 11.90%), although identified as the second most abundant compound, is a simple three-carbon polyol commonly occurring as an oxidative degradation product of glycerol or a non-enzymatic oxidation artifact during sample preparation and chromatographic analysis; its pharmacological contribution is considered negligible. Similarly, the two glycol ether signals detected (peaks 19 and 22; Octaethylene glycol monododecyl ether, 4.55% and 4.08%, respectively; Table 2) are consistent with a non-plant-derived polyethylene-glycol-type surfactant, most likely originating as a residual detergent, extraction-solvent, or labware-related contaminant rather than a genuine constituent of the seed extract; these signals were therefore excluded from further biological interpretation. All ten major constituents reported in Table 2 had NIST library match quality scores of 766 or higher, supporting reasonably confident identification; however, retention index confirmation and comparison with authentic reference standards were not performed for these compounds, and this is acknowledged as a methodological limitation of the present study.

In vitro Antiplasmodial Activity

The antiplasmodial activity of *P. americana* seed methanol extract against *P. falciparum* strain 3D7 was evaluated *in vitro* using a four-parameter logistic (4PL) model (Ritz et al., 2015). Both the extract and chloroquine (CQ) exhibited significant concentration-dependent inhibition of parasite growth (Fig. 2). Chloroquine displayed a steeper dose-response slope, with a faster increase in parasite inhibition at lower concentrations compared with the extract. For each tested concentration, parasite growth was measured in technical triplicate wells within each of three independent biological replicates (n = 3), and the mean ± standard deviation values are reported in Table 3.

Table 3. Percentage of P. Falciparum 3D7 Parasite Growth After Treatment with Avocado Seed (Persea Americana) Methanol Extract and Chloroquine as Positive Control (n = 3)

Concentration (µg/mL)	Avocado Seed Extract % Parasite Growth (Mean ± SD)	Chloroquine % Parasite Growth (Mean ± SD)
100	0.00 ± 0.00	–
10	39.02 ± 0.67	0.00 ± 0.00
1	57.05 ± 0.41	16.01 ± 0.91
0.1	74.23 ± 0.23	27.27 ± 0.36
0.01	87.39 ± 1.14	65.67 ± 0.68
0.001	–	86.04 ± 0.50

Note: SD = standard deviation; n = 3 biological replicates, each performed in technical triplicate. Values represent raw parasite growth (%) relative to the untreated control at 48 h; percentage inhibition equals 100% minus the tabulated growth value at each concentration.

The extract demonstrated progressive growth inhibition across the tested concentration range, from 87.39 ± 1.14% parasite growth at 0.01 µg/mL to complete inhibition (0.00 ± 0.00%) at 100 µg/mL (Table 3). Chloroquine similarly achieved complete inhibition at a markedly lower concentration of 10 µg/mL (0.00 ± 0.00%), consistent with its superior potency as a single-compound antimalarial agent. The extract showed an IC_{50} of 3.41 µg/mL (95% CI: 2.08–5.53 µg/mL), with Hill slope of −0.59 and R^2 = 0.95, suggesting moderate fit to a dose-response. The relatively shallow negative slope is suggestive of gradual concentration-dependent reduction in parasite growth and possibly also due to the complex phytochemical composition of the extract (Alghamdi et al., 2024). Chloroquine, on the other hand, gave an IC_{50} of 0.04 µg/mL (Hill slope: −0.72; R^2 = 0.98), as shown in Table 3, suggestive of the typical potency of a single compound with a well-defined mechanism. The markedly lower IC_{50} of chloroquine relative to the extract is expected given its defined heme polymerization inhibition mechanism.

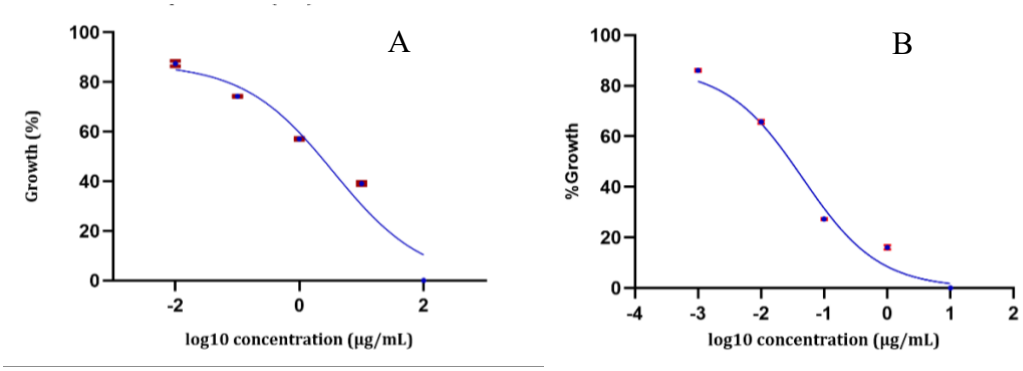


Figure. 2 Four-Parameter Logistic (4PL) Dose–Response Curves of Avocado Seed Extract (A) and Chloroquine for Antiplasmodial Activity Against P. Falciparum 3D7 (B).

Table 3. In Vitro Antiplasmodial Activity of P. Americana Seed Methanol Extract and Chloroquine

Sample	IC_{50} (µg/mL)	95% CI (µg/mL)	Hill Slope	R^2
P. americana seed methanol extract	3.41	2.08–5.53	−0.59	0.95
Chloroquine	0.04	0.03–0.06	−0.72	0.98

Four-parameter logistic (4PL) model; CI is a confidence interval.

The antiplasmodial activity of plant extract is considered potent based on the criteria of the World Health Organization (WHO) with IC50 ≤5 µg/mL (Vergara et al., 2022). The extract with an IC_{50} of 3.41 µg/mL is highly active and suggests efficient recovery of bioactive compounds. This activity may be partly attributable to the flavonoid component identified in the HPLC profile; rutin, quercetin, and kaempferol have, in previous studies of the isolated compounds, been shown to bind key enzymes of the parasite including plasmepsin II, thus inhibiting hemoglobin degradation and suppressing parasite (Olatunde et al., 2025; Suresh et al., 2023). As the present study tested the crude methanol extract rather than isolated constituents, this compound-level attribution remains a plausible hypothesis rather than a demonstrated mechanism, and would need to be verified through bioassay-guided fractionation or testing of the isolated compounds against the parasite. The results collectively suggest that P. americana seed is a promising source of bioactive antiplasmodial compounds worthy of further isolation and mechanistic studies.

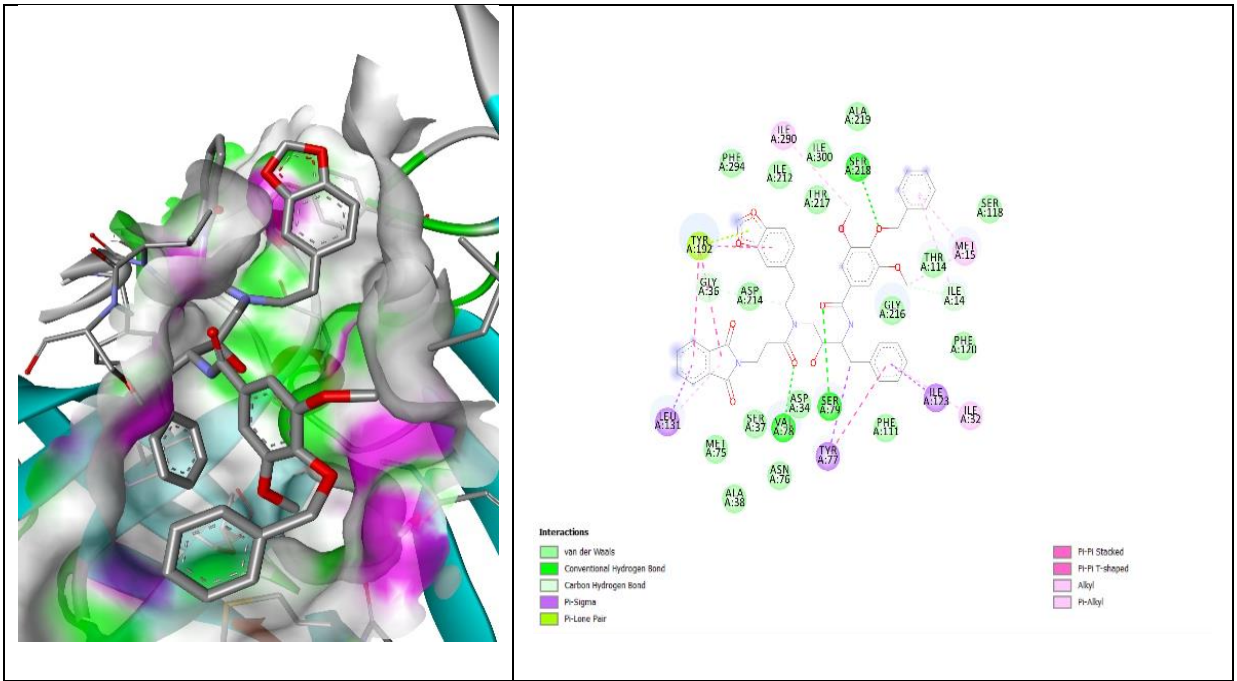
Molecular Docking

Molecular docking simulations were performed to assess the interaction of flavonoid compounds identified from *Persea americana* seeds with plasmepsin II (PDB ID: 1LF3), an aspartic protease involved in the degradation of hemoglobin in the digestive vacuole of *P. falciparum* (Dauda et al., 2026). The docking protocol was validated by redocking the native ligand into the active site of Plasmepsin II and an RMSD value of 2.20 Å was obtained. This value is slightly above the ideal threshold of 2.0 Å, but still within the acceptable validation range for docking studies, indicating that the protocol was able to sufficiently reproduce the orientation of the native ligand (Ramírez & Caballero, 2018). Chloroquine was used as a reference ligand, although its main mechanism of action is through heme polymerization inhibition and not direct Plasmepsin II inhibition, it is the standard comparator in antimalarial studies and its docking behavior against Plasmepsin II has been reported in previous computational studies (Dauda et al., 2026; Olatunde et al., 2025). Chloroquine showed a binding energy of −8.44 kcal/mol. The flavonoid with the highest binding affinity was kaempferol with a docking score of −8.10 kcal/mol, followed by rutin (−7.59 kcal/mol), myricetin (−6.90 kcal/mol), and quercetin (−6.57 kcal/mol). All compounds tested showed binding energies below −6.0 kcal/mol, indicating favorable ligand–protein interactions and potential biological relevance in virtual screening studies (Olafson et al., 2015).

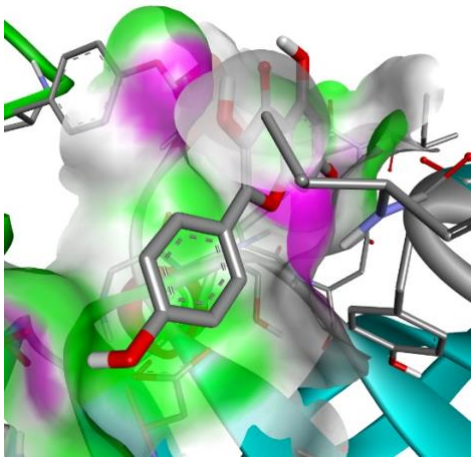
Table 4. Molecular Docking Results of Persea Americana Seed-Derived Flavonoids and Chloroquine Against Plasmepsin II (PDB ID: 1LF3)

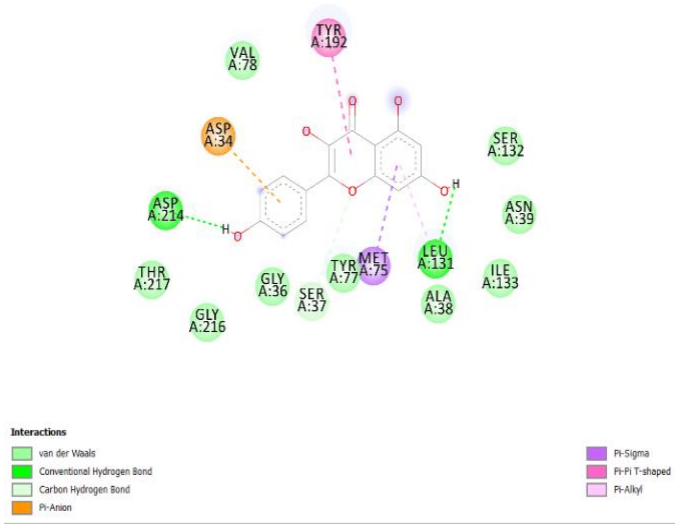
No	Compound	Binding Energy (kcal/mol)	Amino Acid Residues
1	Chloroquine	−8.44	VAL A:78, GLY A:216, TYR A:77, MET A:15, ILE A:32, PHE A:16
2	Kaempferol	−8.10	ASP A:214, LEU A:131, TYR A:192, MET A:75, VAL A:78
3	Rutin	−7.59	HIS A:174, ASN A:173, VAL A:78, ILE A:300
4	Myricetin	−6.90	ASP A:214, TYR A:192, VAL A:78, LEU A:131
5	Quercetin	−6.57	ASP A:214, LEU A:131, TYR A:192

The interaction analysis showed that kaempferol formed hydrogen bonds with ASP A:214 and hydrophobic and π-interactions with TYR A:192, MET A:75 and VAL A:78. The interaction with ASP A:214 is particularly important because this residue is part of the catalytic dyad of Plasmepsin II and plays a crucial role in proteolytic activity (Ji et al., 2022; Nasamu et al., 2020). These results suggest that kaempferol may interfere with substrate binding in the catalytic cleft of the enzyme, which is in agreement with the inhibitory effect observed for a number of flavonoid-based aspartic protease inhibitors (Cheuka et al., 2020; Jin et al., 2014). Rutin was the most abundant flavonoid in the extract but did not show the strongest binding affinity. However, kaempferol, which was less abundant in comparison to rutin, showed greater interaction with Plasmepsin II. This indicates that the binding affinity is more related to the structural compatibility with the active site than the relative abundance of compounds presents within the extract. Overall, the docking results are consistent with the possible contribution of the flavonoid constituents to the antiplasmodial activity of the P. americana seed extract. However, the present findings are predictive and do not take into account the protein flexibility, solvation effects or the possible synergistic effects of the phytochemicals that may be present (Ferreira et al., 2015; Tajuddeen & Van, 2019). Further validation through enzyme inhibition assays and molecular dynamics simulations are required to confirm the stability and the inhibitory potential of the identified compounds.

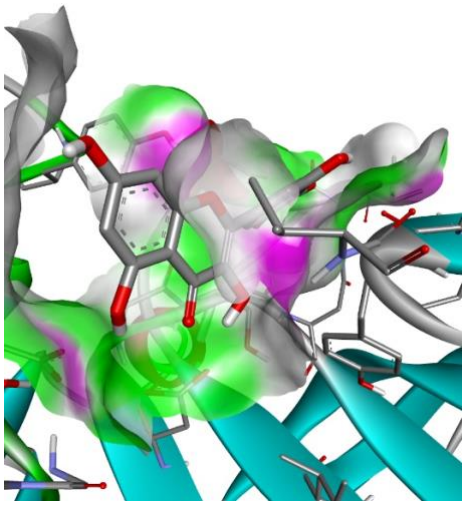


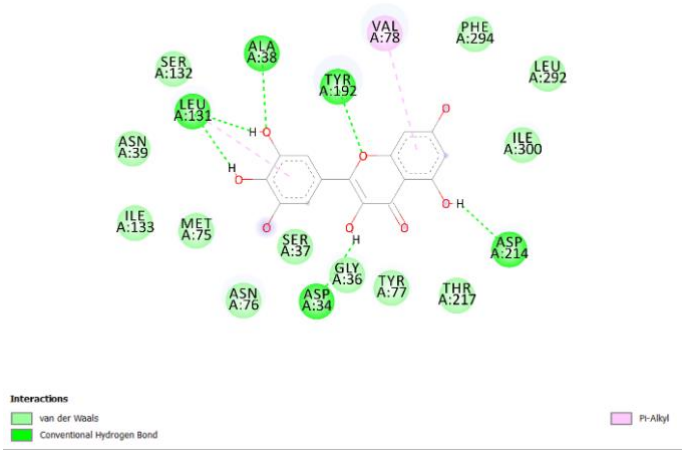
Native Ligand



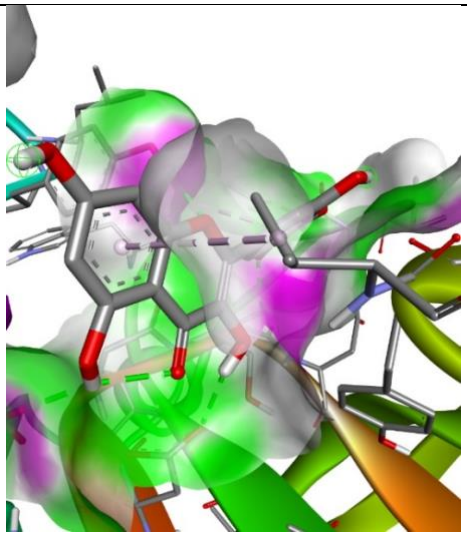


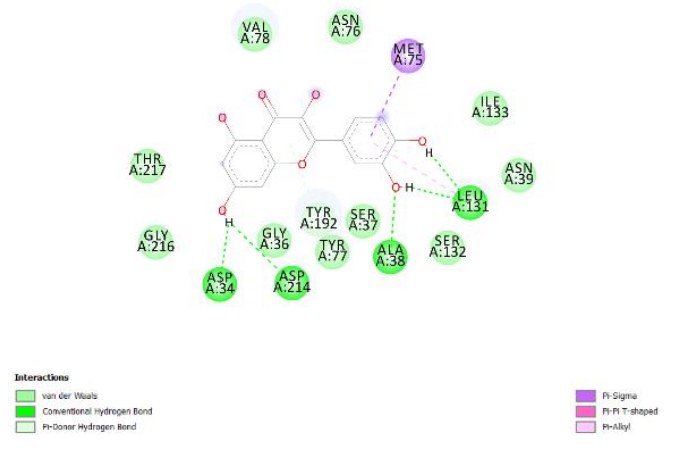
Kaempferol





Myricetin





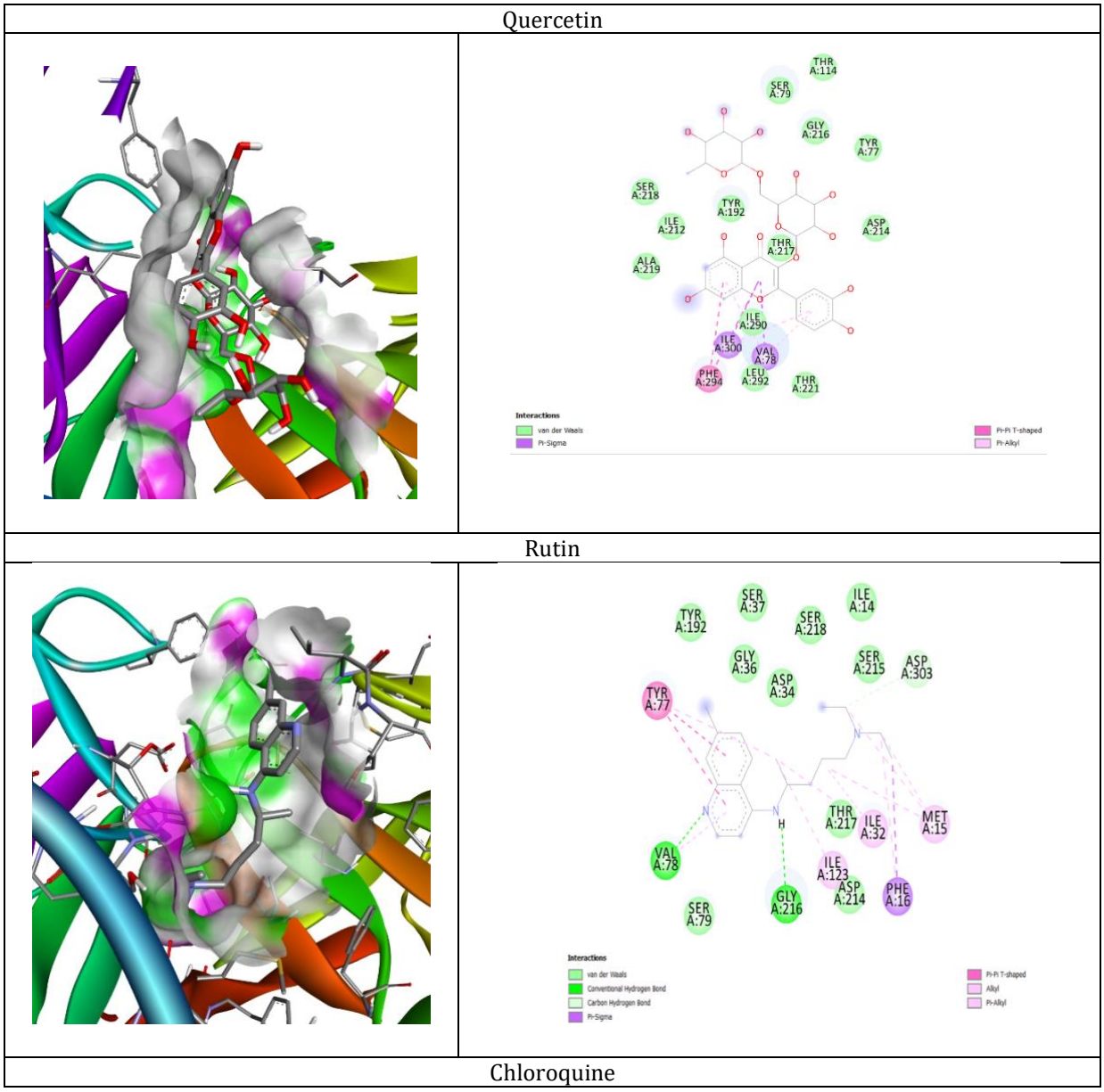


Figure 3. Binding Interaction Patterns of Chloroquine and Avocado Seed-Derived Flavonoids Within the Active Site of Plasmepsin II (PDB ID: 1LF3)

CONCLUSION

The methanolic extract of *P. americana* seeds exhibited good antiplasmodial activity against *P. falciparum* 3D7 (IC_{50} = 3.41 µg/mL). The phytochemical analysis of the extract by HPLC and GC–MS revealed the presence of flavonoids, mainly rutin, furanoid terpenoids and polyunsaturated fatty acids. Molecular docking against plasmepsin II (PDB ID: 1LF3) showed that kaempferol had the strongest binding affinity among the studied flavonoids (−8.10 kcal/mol). However, further studies using isolated compounds are required to determine whether this interaction contributes directly to the antiplasmodial activity observed in the crude extract. Overall, the results suggest that *P. americana* seeds are a promising source of antiplasmodial leads, and kaempferol deserves further study via bioassay-guided isolation, molecular dynamics simulation and *in vivo* validation. Nevertheless, several limitations of this study should be acknowledged: the absence of cytotoxicity data precludes calculation of the Selectivity Index, the RMSD value of 2.20 Å slightly exceeds the ideal docking validation threshold, and the rigid-receptor docking approach does not account for protein flexibility or synergistic interactions among phytochemicals.

ISSN 2776-1150 (Print), ISSN 2776-1169 (online)

SUGGESTION

Future studies should address these limitations to further validate the therapeutic potential of *P. americana* seed-derived compounds.

ACKNOWLEDGMENT

The authors thanks the Director of Integrated Laboratory, Universitas Riau for providing HPLC and GC-MS data as well as Mr. Rahmad Setiawan for supporting in silico analysis. The authors also thanks the staff of the Integrated Laboratory, Universitas Riau, and the Laboratory of Faculty of Mathematics and Natural Sciences, Universitas Muhammadiyah Riau (UMRI), for providing research facilities and technical support during the extraction and phytochemical analysis.

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