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In vitro and molecular docking evaluation of apigenin-7-*O*-glucuronide from *Psorospermum febrifugum* as anti-inflammatory and predicted antiplasmodial compound

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ABSTRACT

Introduction: *Psorospermum febrifugum* is traditionally used in Africa for the treatment of inflammation and malaria. This study aimed to isolate and characterize a bioactive compound from its leaves, evaluate its anti-inflammatory activity, assess the antiplasmodial activity of the CH_2Cl_2/MeOH crude extract, and predict potential molecular targets through docking studies.

Methods: A bioactive compound was isolated from the leaves using chemical, chromatographic, and spectroscopic techniques. Anti-inflammatory activity was evaluated by inhibition of egg albumin and bovine serum albumin (BSA) denaturation. The CH_2Cl_2/MeOH crude extract was tested against chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *Plasmodium falciparum* strains using the SYBR Green assay. Molecular docking of the isolated compound was performed against BSA (4JK4), cyclooxygenase-1 (COX-1), *P. falciparum* lactate dehydrogenase (*Pf*LDH), and dihydroorotate dehydrogenase (*Pf*DHODH).

Results: Apigenin-7-*O*-glucuronide was isolated and is reported here for the first time from *P. febrifugum*. The compound exhibited strong anti-inflammatory activity, with IC_{50} values of 40.61 ± 0.92 and 87.62 ± 0.60 µg/mL against egg albumin and BSA denaturation, respectively. The crude extract showed moderate antiplasmodial activity against 3D7 and Dd2 strains, with IC_{50} values of 44.61 ± 0.08 and 49.08 ± 0.10 µg/mL, respectively. Docking analysis suggested favorable interactions of apigenin-7-*O*-glucuronide with *Pf*LDH, *Pf*DHODH, BSA, and COX-1, indicating potential anti-inflammatory and antiplasmodial mechanisms.

Conclusion: These findings provide scientific support for the traditional use of *P. febrifugum* in treating inflammation and malaria and identify apigenin-7-*O*-glucuronide as a promising bioactive constituent warranting further pharmacological investigation.

Keywords: *Psorospermum febrifugum*, Antiplasmodial, Anti-inflammatory, Molecular docking

Introduction

Inflammation is a protective biological response triggered by tissue and cellular injury caused by pathogens, harmful stimuli, or physical damage. It is characterized by classical signs such as redness, heat, swelling, and loss of function (1). A key event in inflammatory processes is protein denaturation, where structural alterations in tissue proteins, such as egg albumin and bovine serum albumin (BSA), contribute to inflammatory responses (2). Non-steroidal anti-inflammatory drugs (NSAIDs), including diclofenac sodium, are known to prevent protein denaturation and reduce inflammation under pathological conditions (3,4). In addition, phenolic compounds and flavonoids have been reported to exert anti-inflammatory effects through similar mechanisms (5,6). NSAIDs also act by inhibiting cyclooxygenase (COX) enzymes, thereby reducing prostaglandin synthesis, which plays a central role in inflammation (7). The body expresses two main isoforms of this enzyme, COX-1 and COX-2 (8).

Malaria remains a major global health concern, particularly in tropical and subtropical regions of Africa, Latin America, and Asia. In 2023, the World Health Organization reported approximately 263 million cases and over 597,000 deaths worldwide (9). Cameroon is among the most affected countries, with malaria accounting for a significant proportion of mortality, particularly in children under five years old (10). Malaria pathogenesis is strongly associated with inflammatory and oxidative stress responses induced by *Plasmodium* infection of erythrocytes. The parasite stimulates the production of reactive oxygen species (ROS) by host immune

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cells, leading to oxidative stress, lipid peroxidation, and tissue damage, which further amplifies inflammatory responses (11). Because oxidative stress and inflammation are closely linked in malaria pathology, therapeutic strategies targeting both processes are considered promising, particularly those derived from medicinal plants with antioxidant properties (12). Among the validated molecular targets for antimalarial drug development are *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH), a key enzyme in glycolysis essential for parasite energy production, and dihydroorotate dehydrogenase (*Pf*DHODH), which is involved in de novo pyrimidine biosynthesis. Both enzymes are essential for parasite survival and are considered important targets for both chloroquine-sensitive and resistant strains (13). For many years, medicinal plants have been traditionally used to treat many diseases, including malaria (14).

Flavonoid glycosides have been widely reported to exhibit anti-inflammatory activity through interactions with denatured proteins such as BSA and egg albumin, thereby improving their thermal stability. Their biological activity is strongly influenced by structural features, particularly hydroxylation patterns, which affect binding affinity and interaction mechanisms, often involving hydrophobic and hydrogen-bond interactions (15). Flavonoids have also been shown to inhibit COX-1 activity, particularly those containing catechol structures on the B-ring, thereby reducing prostaglandin E2 synthesis (16). In addition, flavonoid glycosides may exert antiplasmodial effects by interacting with PfLDH and PfDHODH, disrupting glycolytic and pyrimidine biosynthesis pathways essential for parasite survival (17,18).

Psorospermum febrifugum Spach (Hypericaceae) is a shrub widely distributed in Africa and used in traditional medicine for the treatment of malaria, inflammatory disorders, skin diseases, ulcers, asthma, and other conditions (19). Previous phytochemical investigations have revealed the presence of flavonoids, phenolics, tannins, terpenoids, glycosides, steroids, and alkaloids, and various pharmacological activities, including antibacterial, anticancer, antiprotozoal, and immunomodulatory effects, have been reported (19). However, despite its ethnomedicinal use in inflammatory diseases, few studies have investigated its anti-inflammatory properties, and to the best of our knowledge, its *in vitro* antiplasmodial activity has not yet been reported. Therefore, this study aimed to isolate secondary metabolites from the CH_2Cl_2/MeOH extract of *P. febrifugum* leaves and to evaluate their anti-inflammatory and antiplasmodial activities both *in vitro* and *in silico*, supported by molecular docking analysis.

Materials and Methods

General experimental procedures

An Avance AMX 500 NMR spectrometer from Bruker was used to record ^1H and ^{13}C NMR spectra, operating at 500 MHz and 125 MHz, respectively, using dimethyl sulfoxide-d_6 (DMSO-d_6) as solvent and tetramethylsilane (TMS) as internal reference standard. A JEOL MS-600H spectrometer was used to record EI-MS spectra. Thin-layer chromatography (TLC analysis on silica gel 60 F_{254} (Merck, Germany) was used to determine the TLC profile. TLC plates were visualized using a UV lamp at 254 and 366 nm and a 10% sulfuric acid solution. The separation process was carried out using column chromatography (CC) on silica gel (60-120 Mesh). The inhibition of denaturation of egg and BSA was determined using a UV-Vis spectrophotometer (Optizen POP, South Korea). The TECAN M 200 microplate reader was used to measure the antiplasmodial activity of the samples by correlating the fluorescence with their ability to inhibit parasite growth.

Chemicals and reagents

Egg albumin, BSA, chloroquine and diclofenac sodium, used as reference compounds, were purchased from Merck (Darmstadt, Germany). Hypoxanthine and Albumax I were purchased from Gibco (Fisher Scientific, USA). Gentamicin and RPMI 1640 medium supplemented with 25 mM HEPES were purchased from Gibco (Fisher Scientific, China) and Gibco (Fisher Scientific, United Kingdom). The Malaria Research and Reference Reagent Resource Center MR4 provided the chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains of *P. falciparum*.

Plant Material

The leaves of *Psorospermum febrifugum* Spach collected in February 2022 in Ngaoundéré, in northern Cameroon (7° 19′ 39″ North, 13° 35′ 04″ East), were identified at the Department of Biological Sciences of the Faculty of Sciences of the University of Ngaoundéré.

Extraction and purification

The leaves of *P. febrifugum* were cleaned, dried, and ground and one kilogram of powdered leaves was macerated in 4 L of CH_2Cl_2/MeOH (1:1) at room temperature (25°C) for 72 h, with stirring. The crude extract was obtained by filtering and evaporating the macerate under vacuum at 40-45°C using a rotary evaporator. The solvent's high extraction capacity justifies its selection for the extraction process. Methanol is used to extract polar compounds (phenols, flavonoids and alkaloids), and dichloromethane for non-polar compounds (terpenoids and fatty acids). The combination of the two solvents allows us to extract a wide range of compounds with different polarities. This extraction procedure was repeated three times using the same plant material. Separation of the CH_2Cl_2/MeOH extract (35 g) of *P. febrifugum* leaves was achieved by silica gel CC (length: 65 cm; internal diameter: 4.5 cm) using a gradient of increasing polarity of ethyl acetate in methanol (1–100 %). Fractionation of this extract yielded 80 fractions (A1-80), which were combined after analysis of the eluates by TLC. Fractions A5-60 were pooled and subjected to CC (length: 25 cm; internal diameter: 2.5 cm) to produce forty subfractions (B1-40). A precipitate formed after the regrouping of the combined subfractions (B8-32) and was then purified by recrystallization in pure methanol to give 18 mg of yellow-orange amorphous powder (1).

Column chromatography was performed using a silica gel column (60–120 mesh, 65 cm length, 4.5 cm internal diameter). Elution was carried out under gravity flow (no forced pressure), using a gradient of ethyl acetate in methanol with

increasing polarity. Fractions were collected in 20 mL tubes. TLC monitoring was performed on silica gel 60 F_{254} plates (Merck), and spots were visualized under UV light at 254 nm and 366 nm, followed by spraying with 10% H_2SO_4 reagent and heating at 105 °C for compound detection.

Qualitative phytochemical analysis

A qualitative phytochemical screening of the CH_2Cl_2/MeOH extract was performed according to standard methods to determine the presence or absence of secondary metabolites. Different phytochemical tests were carried out: phenolic compounds (ferric chloride test), tannins (Gelatin test), flavonoids (Shinoda test), alkaloids (Mayer and Dragendorff reagents), terpenoids, sterols (Liebermann-Burchard test), glycosides (Keller-Killiani test), and saponins (foam index) (20).

Inhibition of egg albumin Denaturation

The slightly modified method of Mirke *et al*. was used for the egg albumin denaturation inhibition test of the extract and the isolated compound (21). Four different concentrations (25-150 µg/L) of CH_2Cl_2/MeOH extract and the isolated compound were prepared by dissolution in methanol. 2 mL of each sample, 0.2 mL of egg albumin (1%), and 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) were mixed. The resulting mixtures were incubated at 37 ± 2°C for 15 minutes and then heated to 70°C for 5 minutes. The control solution consisted of the reaction mixture containing egg albumin and phosphate-buffered saline without any plant extract or standard drug, treated under identical experimental conditions. Diclofenac sodium serves as the positive control. A UV-visible spectrophotometer was used to measure absorbance at 660 nm.

$$\text{Denaturation inhibition}(\%) = ((A_0 - A_1)/A_0) \times 100$$

Where A_0 represents the absorbance of the control solution and A_1 that of the plant material or standard. The sample concentration that showed IC_{50} (50% inhibition was determined from the interpolation of the linear regression analysis.

Inhibition of BSA Denaturation

The method described by Dernouich et al. (22), slightly modified and based on the inhibition of denaturation by BSA, was used to evaluate the anti-inflammatory activity of the crude extract and the isolated compound of *P. febrifugum*. A mixture of 50 µL of the CH_2Cl_2/MeOH extract or the isolated compound dissolved in methanol at various concentrations (10–150 µg/mL) and 450 µL of BSA (1%) was incubated for 20 minutes at 37 °C. Then, the mixture was heated for 3 minutes at 57°C, cooled, and 2.5 mL of phosphate buffer was added. The control solution is the mixture without plant material. The absorbance of the samples was measured using a UV-visible spectrophotometer at 660 nm. Diclofenac sodium was used as a positive control. All tests were performed in triplicate. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\text{Denaturation inhibition}(\%) = ((A_C - A_S)/A_C) \times 100$$

Where A_C represents the absorbance of the control solution, and A_S that of the plant material or standard. The sample concentration showing 50% inhibition (IC_{50}) was determined by interpolation from linear regression analysis.

In vitro antiplasmodial assay

The antiplasmodial activity of *P. febrifugum* leaf extract was evaluated *in vitro* using chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains of *P. falciparum*. The method described by Trager and Jensen (23) was used for the culture of *P. falciparum*. Cultures were kept in fresh O+ human blood cells suspended at 4% haematocrit in complete RPMI 1640 medium with (25 mM HEPES, 0.5% albumax, 45 µg/L hypoxanthine, and 50 µg/L gentamicin), and incubated at 37°C in a humidified atmosphere with 5% CO_2. The replacement of the culture medium was done on a daily basis with fresh complete medium until parasitemia reached 1–2%.

The antiplasmodial activity of the CH_2Cl_2/MeOH extract mixture was assessed using the SYBR Green I fluorescence assay described by Smilkstein *et al*. (24). The method described by Lambros and Vanderberg (25) was used to obtain ring-stage synchronized parasites by treatment with 5% (w/v) sorbitol before each experiment. Dimethyl sulfoxide was used as a dilution solvent to prepare different concentrations of dry extract and chloroquine, the reference drug (0-500 µg/mL). A 72 h incubation at 37°C in microwells was performed for sorbitol-synchronized parasites under normal culture conditions at 1% hematocrit and 2% parasitemia in the presence of prediluted extract and artemisinin (10 µL). After incubation, 100 µL of SYBR Green I buffer (6 µL of 10,000 × SYBR Green I (Invitrogen) + 600 µL of red blood cell lysis buffer {Tris (25 mM; pH 7.5)} + 360 µL of EDTA (7.5 mM) + 19.2 µL of parasite lysis solution {saponin (0.012%; w/v)} + 28.8 µL of Triton X-100 (0.08%; v/v)}) was added to each well, mixed gently twice with a multichannel pipette, and incubated in the dark at 37°C for 1h. The measurement of fluorescence was carried out using a TECAN M 200 microplate reader, which has excitation and emission settings set at 485 and 538 nm, respectively. Fluorescence counts were plotted against sample concentration to determine the IC_{50} (50% inhibitory concentration) by dose-response curve analysis. Experiments were performed in triplicate. Validation of the results was performed microscopically by examining Giemsa-stained blood spots for each extract and control.

Molecular Docking analysis

The Molecular Operating Environment software (MOE 2013.08; Chemical Computing Group, Montreal, QC, Canada) (26) was used for the molecular docking of compound **1** on the active site of two target proteins, BSA and COX-1, linked to anti-inflammatory activities and two others linked to antiplasmodial activity (*Pf*LDH and *Pf*DHODH). The crystal structures of BSA (PDB identifier: 4JK4, resolution: 2.65 Å), COX-1 (PDB identifier: 3N8Y, resolution: 2.60 Å), *Pf*LDH (PDB identifier: 2X8L, resolution: 1.60 Å) and *Pf*DHODH (PDB identifier: 6GJG, resolution: 1.99 Å) were downloaded from the Protein Data Bank (https://www.rcsb.org/). The removal of water molecules and heteroatoms from the proteins was carried out.

The selected ligands (apigenin-7-*O*-glucuronide (**1**), diclofenac sodium and chloroquine) and the reference ligand were obtained from the chemical database (https://pubchem.ncbi.nlm.nih.gov/) and loaded into MOE. The ligands were then subjected to 3D protonation and energy minimization. The MMFF94X force field was used to minimize the energy of the ligands and the protein structure. The best conformation was determined after docking based on the S score and the interacting residues (27).

The docking protocol was validated by redocking the co-crystallized ligands into the active sites of the target proteins. The root mean square deviation (RMSD) values between the docked and crystallographic poses were found to be < 2.0 Å (4JK4: 0.76 Å; 3N8Y: 0.79 Å; 2X8L: 1.62 Å; 6GJG: 1.69 Å), confirming the reliability of the docking procedure.

ADMET and pharmacokinetic predictions

Following the molecular docking study, the physicochemical properties and ADMET (Absorption, Distribution, Metabolism, Elimination and Toxicity) of compound **1** and the reference ligand were examined using the online tool Online (28).

Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean ± SEM. Statistical analyses were performed using SPSS version 21. Differences between groups (CH_2Cl_2/MeOH extract, compound **1**, and reference drug) were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results

Phytochemical screening

Extraction of *P. febrifugum* leaves yielded 117.0 g (11.7 %) of CH_2Cl_2/MeOH (1:1) extract. Phytochemical analysis of this extract revealed the presence of flavonoids, alkaloids, terpenoids, steroids, glycosides, and phenolic compounds. However, tannins were not detected in this extract.

Compound isolation

Separation of the CH_2Cl_2/MeOH extract by silica gel column chromatography using an EtOAc/MeOH gradient of increasing polarity gave compound **1**, a yellow-orange amorphous solid soluble in DMSO. The structure of Compound **1** was elucidated by one- and two-dimensional nuclear magnetic resonance and mass spectrometry. High-resolution mass spectrum (HRESIMS) at *m/z* 446.08 (calculated for $C_{21}H_{18}O_{11}$). ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) (Table 1). Its ^{1}H NMR data showed two doublets at δ_H 7.81 (J = 7.5 Hz) and 6.92 (J = 7.5 Hz), characteristic of an AA'BB' system of two pairs of ortho-coupled aromatic protons, suggesting the presence of a symmetrical aromatic ring. A two-proton meta-coupled AB system is also observed, with resonance signals at δ_H 6.45 (1H; d; J = 1.9 Hz; H-8) and 6.81 (1H; d; J = 1.9 Hz; H-6). Two singlet protons are detected at δ_H 12.94 and 6.80, corresponding respectively to a chelated hydroxyl group at position 5 and H-3 of the flavone's C ring. The fully decoupled ^{13}C-NMR spectrum displayed signals from 21 carbon atoms. The DEPT-135 and HSQC spectra revealed nine quaternary carbons and twelve sp^2 hybridized methines, five of which were oxygenated, including a carbonyl group. These data, compared with those in the literature, allowed us to identify compound **1** as apigenin 7-*O*-glucuronide (Fig. 1), previously described in the aerial parts of *Agrimonia pilosa* (29). This compound is reported here for the first time in this species.

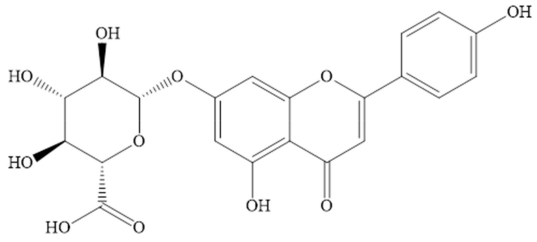

FIGURE 1 - Structure of apigenin 7-*O*-glucuronide (1).

TABLE 1 - ^{1}H (500 MHz) and ^{13}C (125 MHz) nuclear magnetic resonance data for compound **1** in DMSO-d_6

Position	1		Published (29)
	δ_C	δ_H (m, J (Hz))	δ_C
1	-	-	-
2	164.4	-	165.1
3	103.3	6.81 (s)	102.8
4	181.9	-	181.4
10	105.3	-	105.9
5	161.4	-	162.1
6	99.9	6.45 (d, 1.9)	100.2
7	162.9	-	162.5
8	94.9	6.80 (d, 1,9)	95.3
9	157.7	-	157.6
1′	121.1	-	120.8
2′6′	128.9	7.81 (d, 7.5)	129.3
3′; 5′	116.4	6.92 (d, 7.4)	116.7
4′	161.1	-	159.9
1″	99.5	5.18 (d, 7.7)	99.5
2″	73.3	3.33 (m)	73.7
3″	76.5	3.37 (d, 7.7)	77.2
4″	72.1	3.35 (m)	72.3
5″	75.1	3.85 (m)	74.4
6″	171.7	-	172.6

Anti-inflammatory activity

The anti-inflammatory properties of the CH_2Cl_2/MeOH extract and compound **1** were assessed by observing the effect on the denaturation of egg and BSA caused by heat treatment. The egg albumin denaturation inhibition test, evaluated at five doses (25-150 µg/mL), showed a

dose-dependent inhibition (Table 2). Compound **1** is active in this inhibition (IC_{50} = 40.61 ± 0.92 µg/mL) as well as the CH_2Cl_2/MeOH extract (IC_{50} = 79.95 ± 0.40 µg/mL). The maximum inhibition was observed at 79.52 ± 1.07% for compound **1** and 59.47 ± 0.29 % for the CH_2Cl_2/MeOH extract at the concentration of 150 µg/mL.

TABLE 2 - Inhibition of egg albumin denaturation of *P. febrifugum* extract and compound **1**

Samples	Conc (µg/mL)	Inhibition ± SEM (%)[a]	IC_{50} (µg/mL)
CH_2Cl_2/MeOH extract	10	26.22±0.41[a]	79.94±0.40[a]
	25	31.32±0.28[b]	
	50	39.82±0.01[d]	
	100	56.82±0.21[g]	
	150	73.82±0.29[h]	
Apigenin-7-*O*-glucuronide (**1**)	10	35.43±0.16[c]	40.63±0.92[b]
	25	42.56±0.17[e]	
	50	54.47±0.14[f]	
	100	78.27±0.15[j]	
	150	100.0±0.0[k]	
Diclofenac sodium	10	43.39±0.04[e]	15.92±0.34[c]
	25	55.84±0.16[f,g]	
	50	76.24±0.04[i]	
	100	100.0±0.0[k]	
	150	100.0±0.0[k]	

Values are expressed as Mean±SEM in triplicate experiments. Values with different superscript letters in the same column are significantly different (p<0.05; ANOVA with Tukey's post hoc test).

BSA denaturation inhibition assay was evaluated at five doses (25–200 µg/mL) and showed concentration-dependent inhibition (Table 3). Compound **1** showed strong inhibition of BSA denaturation, with an IC_{50} value of 87.62 ± 0.6 µg/mL, compared with the CH_2Cl_2/MeOH extract (IC_{50} = 138.68 ± 0.36 µg/mL). At a concentration of 200 µg/mL, compound **1** showed a maximum inhibition of 80.3 ± 1.07%, while that of the CH_2Cl_2/MeOH extract was 65.05 ± 0.17%. As for the standard anti-inflammatory drug, diclofenac sodium, it showed 100% inhibition at this concentration.

Antiplasmodial activity

The CH_2Cl_2/MeOH (1:1) extract of *P. febrifugum* leaves was evaluated for antiplasmodial activity against the chloroquine-sensitive 3D7 strain and the chloroquine-resistant Dd2 strain of *P. falciparum*. The extract exhibited IC_{50} values of 49.08 ± 0.10 µg/mL and 44.61 ± 0.08 µg/mL against the 3D7 and Dd2 strains, respectively. The corresponding values obtained for chloroquine, used as the reference drug, are presented in Table 4.

In silico docking study

Molecular docking of apigenin-7-*O*-glucuronide (**1**) was used to study its binding to BSA and COX-1 and compare this to diclofenac sodium, a drug used to treat inflammation. The inhibitory activities of apigenin-7-*O*-glucuronide (**1**) on BSA, COX-1, *Pf*LDH and *Pf*DHODH were evaluated based on their minimum binding energies associated with complex formation at the catalytic activity level. Apigenin-7-*O*-glucuronide (**1**) exhibited a minimum binding energy of -6.99 kcal/mol for BSA and -7.72 kcal/mol for COX-1 (Table 5). Compound **1** showed the highest docking scores compared to the

TABLE 3 - Inhibition of BSA denaturation of *P. febrifugum* extract and compound **1**

Samples	Conc (µg/mL)	Inhibition ± SEM (%)	IC_{50} (µg/mL)
CH_2Cl_2/MeOH extract	25	21.59±0.11[a]	138.68±0.36[a]
	50	27.83±0.03[b]	
	100	40.25±0.13[d]	
	150	52.40±0.29[f]	
	200	65.33±0.45[g]	
Apigenin-7-*O*-glucuronide (**1**)	25	34.97±0.23[c]	87.62±0.60[b]
	50	40.98±0.54[d]	
	100	52.97±0.49[f]	
	150	64.97±0.99[g]	
	200	76.97±0.07[h]	
Diclofenac sodium	25	46.55±0.10[e]	29.93±0.23[c]
	50	64.06±0.03[g]	
	100	99.05±0.05[i]	
	150	100.0±0.0[i]	
	200	100.0±0.0[i]	

Values are expressed as Mean ± SEM in triplicate experiments. Values with different superscript letters in the same column are significantly different (p<0.05; ANOVA with Tukey's post hoc test).

TABLE 4 - *In vitro* antiplasmodial activity of *P. febrifugum* extract

Samples	IC_{50} (µg/mL)	
	***Pf* 3D7**	***Pf* Dd2**
CH_2Cl_2/MeOH (1:1) extract	49.08±0.10	44.61±0.08
Chloroquine (nM)	21.65±0.20	27.22±0.02

complexes formed with diclofenac sodium, the reference ligand. The binding mode observed between apigenin-7-*O*-glucuronide (**1**) and the residues of the BSA active site reveals the formation of a proton-acceptor bond, characteristic of strong interactions, and was observed with residue Arg256 (Fig. 2). Similarly, an ionic bond was observed with Lys221. The complex formed between COX-1 and apigenin-7-*O*-glucuronide (**1**) exhibited two acceptor hydrogen bonds with residues Gln 203 and His207 (Fig. 2).

Molecular docking prediction of the inhibitory activity of apigenin-7-*O*-glucuronide (**1**) against *Pf*LDH (2X8L) and *Pf*DHODH (6GJG) revealed minimum binding energies of -4.29 kcal/mol and -9.82 kcal/mol, respectively. These docking scores are higher than those of complexes formed with chloroquine, the reference drug (Table 6). For 2X8L, compound **1** exhibits fewer interactions with residues (Fig. 2). Two strong interactions, including an H-acceptor interaction and an ionic interaction, are observed with residue Arg185. In contrast, compound **1** interacts with many residues within the active site of 6GJG, notably through strong hydrogen bonds (hydrogen acceptor) with residues Asn342 (2.99 Å) and Lys229 (2.64 Å), and weak π-H bonds with Ser529 (4.32 Å) and π-π with Tyr528 (3.84 Å) (Fig. 2).

ADMET and pharmacokinetic properties

The evaluation of the pharmacokinetic parameters of apigenin 7-*O*-glucuronide, including its intestinal absorption and cerebral permeation, constitutes a key toxicokinetic parameter that determines its toxicity, particularly its neurotoxicity. Predictions of gastrointestinal absorption of the isolated compound were determined using the Lipinski, Ghose, Veber, and Muegge rules (30-33).

The pharmacokinetic evaluation of compound **1** revealed a mixed drug-likeness profile. Although the compound complies with Ghose's rule, it violates Lipinski's rule (two violations: hydrogen bond donors > 5 and hydrogen bond acceptors > 10), Veber's rule (TPSA > 140 Å²), and Muegge's rule, reflecting its high polarity and relatively large molecular size. These rules are widely used to predict oral drug-likeness and gastrointestinal absorption potential of small molecules (31-33).

SwissADME predictions (Table 7) indicate low gastrointestinal absorption and no blood–brain barrier permeability, suggesting limited oral bioavailability under conventional administration routes (28). However, the compound does not inhibit major cytochrome P450 isoforms, suggesting a low risk of metabolic drug–drug interactions (SwissADME prediction) (28). Its physicochemical profile suggests that formulation strategies such as nanocarriers or prodrug approaches may be required to improve bioavailability, as reported for other poorly permeable flavonoid glycosides (34,35).

Discussion

Phytochemical analysis of CH_2Cl_2/MeOH extract revealed the presence of flavonoids, alkaloids, terpenoids, steroids, glycosides, and phenolic compounds, but no trace of tannins. This absence of detectable tannins in the present study contrasts with previous reports on *P. febrifugum* leaf extracts (36). Such discrepancies may be attributed to variations in environmental and ecological conditions, including geographical location, climatic factors, harvest season, and plant physiological stage, all of which are known to influence the biosynthesis and accumulation of secondary metabolites. In addition, differences in phytochemical screening methodologies may also affect the detection of tannins. The collection of plant material in Ngaoundéré during February may therefore have contributed to the observed phytochemical profile.

The anti-inflammatory activity of the extract and compound **1** was demonstrated by studying the inhibition of egg albumin and BSA denaturation. Denaturation of proteins such as egg albumin and BSA, leading to the loss of most of their biological functions, is one of the causes of inflammation (2). Phenolic compounds such as flavonoids have been previously shown to prevent the denaturation of proteins such as egg albumin and BSA by interacting (22,37). Thus, flavonoids may interact with these proteins to improve their thermal stability. Compound **1**, a flavone glycoside, showed inhibition of egg albumin and BSA denaturation. These results corroborate several previous studies that demonstrated the interaction between flavonoids and proteins such as egg albumin and BSA to improve their thermal stability. The chemical structure of flavonoids influences their interactions with proteins. Indeed, it was shown that hydroxyl groups present on flavonoid rings can increase the thermal stability of proteins (37). This could explain the anti-denaturation activity of albumin and BSA observed for compound **1**. A previous study showed that Flavonoids (chrysin and umbelliferone) isolated from *Potentilla evestita* showed inhibition of protein denaturation activity with IC_{50} values of 119 and 112 µg/mL, respectively (38).

The CH_2Cl_2/MeOH extract of *P. febrifugum* exhibited antiplasmodial activity with IC_{50} values ranging from 44.61 to 49.08 µg/mL against the chloroquine-resistant *Pf*Dd2 and chloroquine-sensitive *Pf*3D7 strains of *P. falciparum*. The classification proposed by Kumari *et al*. (39) was used to interpret the IC_{50} values (µg/mL): very active ($IC_{50} < 5$), active ($5 \leq IC_{50} < 50$), moderately active ($50 \leq IC_{50} \leq 100$), and inactive ($IC_{50} > 100$). According to this classification, the extract falls within the active category against both parasite strains. However, because the IC_{50} values are close to the upper limit of this category, the extract may be considered to exhibit moderate but promising antiplasmodial activity, warranting further phytochemical and pharmacological investigations. The slightly lower IC_{50} observed for the Dd2 strain compared to the 3D7 strain suggests a marginally higher apparent activity against the resistant strain. However, given the small difference between the values and the standard deviations obtained from triplicate experiments,

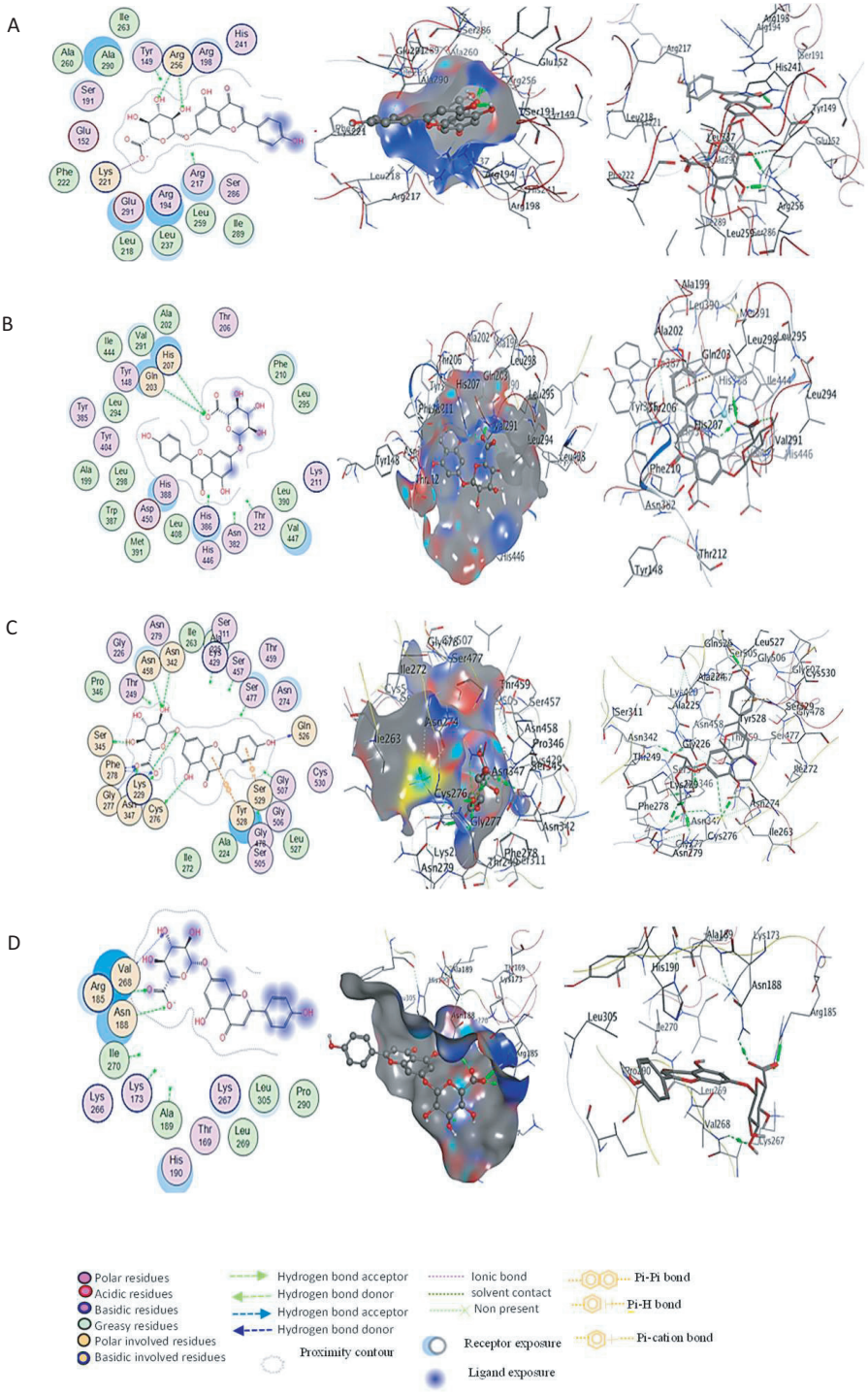

FIGURE 2 - 2D and 3D visualizations of the best pose of apigenin-7-*O*-glucuronide_proteins for docking using MOE. (A) Binding mode of apigenin-7-*O*-glucuronide (1) in the active site of BSA (PDB ID: 4JK4), showing hydrogen bond interactions (dashed lines) with key residues such as Arg256 and Lys221, along with hydrophobic interactions stabilizing the ligand within the binding pocket. (B) Binding mode of compound **1** in the active site of BSA (PDB ID: 3N8Y), showing hydrogen bond interactions (dashed lines) with key residues such as Gly203 and His207, along with hydrophobic interactions stabilizing the ligand within the binding pocket. (C) Binding mode of apigenin-7-*O*-glucuronide in the active site of BSA (PDB ID: 2X8L), showing hydrogen bond interactions (dashed lines) with key residues such as Arg185, Asn188 and Val268, along with hydrophobic interactions stabilizing the ligand within the binding pocket. (D) Binding mode of **1** in the active site of BSA (PDB ID: 6GJG), showing hydrogen bond interactions (dashed lines) with key residues such as Gly277, Ser529, Asn347, Ser345, Phe278, Tyr528, Lys229, Cys276, Gln526, Asn342 and Asn458, along with hydrophobic interactions stabilizing the ligand within the binding pocket.

TABLE 5 - Docking results of the isolated compound and standard ligand (diclofenac sodium) into BSA protein (4JK4) and COX-1 enzyme (3N8Y)

Interactions	AC	IRR	TIB	Distance Å	Docking Score (kcal/mol)
1 and BSA	O 3	Arg 256	H-acceptor	3.05	–6.99
	O 5	Arg 256	H-acceptor	2.85	
	O 9	Lys 221	Ionic	2.94	
DS and BSA	N-14	Arg217	H-donor	2.97	–4.99
	CL-10	Arg256	H-acceptor	2.95	
1 and COX 1	O-9	Gln 203	H-acceptor	2.92	–7.72
	O-9	His 207	H-acceptor	2.74	
	O-9	His 207	Ionic	2.74	
DS and COX 1	6-ring	Gln203	pi-H	3.93	-6.15

BSA – bovine serum albumin, COX 1 – cyclooxygenase 1, AC – atom of compound, IRA – involved receptor atoms, IRR – involved receptor residues, TIB – types of interaction bonds, 1 – apigenin 7-*O*-glucuronide, DS – diclofenac sodium.

TABLE 6 - Docking results of the isolated compound and standard ligand (chloroquine) into *Pf*LDH (2X8L) and *Pf*DHODH (6GJG) proteins

Interactions	AC	IRR	TIB	Distance Å	Docking Score (kcal/mol)
1 and *Pf*LDH	O 4	ALA 189	H-acceptor	3.32	–4.29
	O 9	ASN 188	H-acceptor	3.17	
	O 10	ARG 185	H-acceptor	2.84	
	O 10	ARG 185	H-acceptor	3.27	
	O 10	ARG 185	ionic	2.84	
	O10	ARG 185	ionic	3.27	
CQ and *Pf*LDH	N 4	ARG 185	H-acceptor	3.48	–4.05
1 and *Pf*DHODH	O 7	SER 345	H-donor	3.19	–9.82
	O 12	CYS 276	H-donor	3.51	
	O 14	GLN 526	H-donor	2.69	
	O 2	LYS 229	H-acceptor	2.79	
	O 5	ASN 342	H-acceptor	2.99	
	O 5	ASN 458	H-acceptor	2.89	
	O 9	LYS 229	H-acceptor	2.64	
	O 9	PHE 278	H-acceptor	3.00	
	O 10	GLY 277	H-acceptor	2.70	
	O 10	PHE 278	H-acceptor	3.25	
	O 10	ASN 347	H-acceptor	3.23	
	O 9	LYS 229	H-acceptor	2.64	
	6-ring	SER 529	Pi-H	4.32	
	6-ring	TYR 528	Pi-pi	3.84	
CQ and *Pf*DHODH	N 4	LYS 229	H-acceptor	3.01	–8.65

*Pf*LDH- *Plasmodium falciparum* lactate dehydrogenase, *Pf*DHODH – *Plasmodium falciparum* dihydroorotate dehydrogenase, AC – atom of compound, IRA – involved receptor atoms, IRR – involved receptor residues, TIB – types of interaction bonds, 1 – apigenin 7-*O*-glucuronide, CQ – chloroquine.

TABLE 7 - Drug-likeness properties of compound **1** and standard ligand (diclofenac sodium)

	Apigenin 7-*O*-glucuronide	**Diclofenac sodium**
Lipinski	No; 2/NorO>10 NHorOH>5	Yes
Ghose	Yes	Yes
Veber	No; 1/TPSA>140	Yes
Egan	No; 1/TPSA>131.6	Yes
Muegge	No; 3/TPSA>150 H-acc>10 H-don>5	Yes
Molecular Weight (g/mol)	446.36	318.13
Total number of atoms	50	30
Number of rotatable bonds	4	4
Number of H-bond acceptors	11	2
Number of H-bond donors	6	1
TPSA $Å^2$	187.12	52.16
Consensus Log $P_{o/w}$	0.17	0.03
Molar Refractivity	106.72	310
Bioavailability score	0.11	0.55
Synthetic accessibility	5.06	2.25
Solubility (log mol/L)	-2.76	-4.64
BBB	No	Yes
HIA	Low	High
P-gp substrate	Yes	No
CYP1A2 inhibitor	No	No
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	No

H-acc: hydrogen bond acceptors, H-don: hydrogen bond donors, N or O: number of oxygen atoms, NH or OH: number of oxygen atoms bonded to hydrogen atoms, TPSA: topological polar surface, consensus Log $P_{o/w}$: consensus lipophilicity, BBB: blood-brain barrier, HIA: human gastrointestinal absorption, P-gp: P-glycoprotein.

this variation is not statistically significant and should not be interpreted as a meaningful difference in strain sensitivity. When compared to other medicinal plant extracts reported in the literature, this level of activity is consistent with several crude extracts traditionally used for malaria treatment, which often exhibit IC_{50} values in the range of 20–100 µg/mL before fractionation (40-43). Such moderate activity is commonly observed in complex plant extracts where synergistic and antagonistic interactions between phytochemicals may influence overall efficacy. Although this potency is not sufficient for direct therapeutic application, it is considered relevant for further bio-guided fractionation, which may lead to the isolation of more active constituents with improved antiplasmodial profiles. Furthermore, flavonoid glycosides such as vogeloside A and 3,7,4'-trihydroxy-3'-(4-hydroxy-3-methylbutyl)-5,6-dimethoxyflavone isolated from *Duranta repens* showed potent antiplasmodial activity with IC_{50} values of 12.3±1.4 µM (*Pf*chloroquine-sensitive) and 12.9±1.6 µM (*Pf*chloroquine-resistant), 5.2±1.7 µM (*Pf*chloroquine-sensitive) and 5.9±1.4 µM (*Pf*chloroquine-resistant), respectively (44). These findings support the traditional use of *P. febrifugum* in the treatment of malaria. The CH_2Cl_2/MeOH extract's potency against the chloroquine-resistant (Dd2) strain indicates that it might serve as an effective cure for chloroquine-resistant malaria. The yield of

compound **1** was too low to permit an *in vitro* antiplasmodial assay; therefore, molecular docking analysis was performed to provide preliminary evidence of its antiplasmodial activity.

The results of molecular modelling confirmed the interactions of compound **1** with the active sites of the proteins BSA (4JK4) and COX-1 (3N8Y). Specifically, the interactions between compound **1** and COX-1 are primarily weak non-covalent interactions, including H-acceptor bonds with residues Gln203 and His207. The interactions between compound **1** and BSA are primarily an H-acceptor bond formed with residue Arg256. These interactions between the two proteins and the compound lead to the formation of stable complexes with similar interaction energies, indicating that the isolated compound is an inhibitor of BSA and COX-1. Furthermore, diclofenac sodium, an anti-inflammatory drug, exhibited significantly higher binding energies for BSA and COX-1, demonstrating that the tested compound exhibited a more favourable (lower) binding energy, indicating a stronger interaction with the target proteins. The interactions between BSA and compound **1**, a flavone glycoside, stabilize the structure and prevent denaturation of BSA, illustrating the anti-inflammatory properties of compound **1** (2). These interactions could be due to the presence of hydroxyl groups on rings A, B, and C (15) of the flavone glycoside. These results are consistent with previous studies that demonstrated interactions between three flavonoids (eriodictioside, glabridin, and vitexicarpine) and BSA in molecular models (45). Similarly, flavonoids isolated from the leaves of *Onopordum acanthium* L. have shown increased COX-1 inhibitory activity *in silico* (46). The tested compound interacted with the COX-1 enzyme by forming hydrogen bonds with the amino acids in its active site, thereby blocking the enzyme's access to its substrate. Previous studies show that flavonoids' hydroxyl groups bind to COX-1 via hydrogen bonds, resulting in reversible inhibition. The molecular docking results corroborate the *in vitro* tests, which showed significant anti-denaturation activity against BSA (IC_{50} = 15.24 ± 0.32 µg/mL), compared to diclofenac sodium, the reference drug (IC_{50} = 31.45 ± 0.20 µg/mL). The apigenin-7-*O*-glucuronide ligand (**1**) interacted with residue *Pf*DHODH and other residues of the active site and shared interactions with the co-crystallization ligand. This indicates that compound **1** was strongly bound to the enzyme via hydrogen bonds with residue Asn342 (2.99 Å), a weak π-H bond with residue Ser529 (4.32 Å), and a weak π-π bond with residue Tyr528 (3.84 Å). Since the interactions of compound **1** with the active site of *Pf*LDH were less important than those with the active site of *Pf*DHODH, this compound was better localized on the active site of *Pf*DHODH (-9.82 kcal/mol) than on that of *Pf*LDH (-4.29 kcal/mol). Compound **1** interacts less strongly with the active site of *Pf*LDH than with that of *Pf*DHODH and therefore localizes more readily to the active site of *Pf*DHODH than to that of *Pf*LDH (-4.29 kcal/mol). This difference in affinity between the compound and the two proteins is explained by the difference in its mechanism of action on each of them. *Pf*LDH blocks anaerobic glycolysis in the parasite, while *Pf*DHODH is involved in the de novo biosynthesis of pyrimidines, notably the oxidation of dihydroorotate to orotate, a precursor of pyrimidines (13). *Pf*DHODH is a promising enzymatic target for the development of antimalarial drugs, while *Pf*LDH is used for rapid diagnostic tests for malaria. Both proteins are enzymatic targets for evaluating compound interactions in chloroquine-sensitive and chloroquine-resistant *Plasmodium* strains. The difference between the two strains is a gene mutation (*Pf*CRT) (47). Flavonoids inhibit *Pf*LDH and *Pf*DHODH, glycolytic and mitochondrial enzymes, respectively, thereby circumventing the resistance mechanisms associated with the *Pf*CRT pump (18). The interactions between flavonoids and the *Pf*LDH and *Pf*DHODH proteins are due to the presence of OH groups on rings A, B, and C. This could explain the interactions between compound **1** and these protein targets, thus validating the *in silico* inhibition of its antiplasmodial activity.

Although apigenin-7-*O*-glucuronide (**1**) exhibited strong binding affinity toward *Pf*DHODH in the molecular docking analysis, its direct antiplasmodial activity against *P. falciparum* strains was not evaluated because the quantity of purified compound obtained was insufficient for additional biological assays. Therefore, the docking results should be interpreted as preliminary evidence of potential antiplasmodial activity. Future studies involving larger-scale isolation or synthesis of the compound are necessary to validate these findings through *in vitro* and *in vivo* antiplasmodial investigations.

The reliability of the molecular docking results was supported by validation of the docking protocol through redocking of the co-crystallized ligands into the active sites of the target proteins. The obtained RMSD values of 0.76 Å (PDB ID: 4JK4), 0.79 Å (PDB ID: 3N8Y), 1.69 Å (PDB ID: 6GJG) and 1.62 Å (PDB ID: 2X8L) were well below the commonly accepted threshold of 2.0 Å, indicating accurate reproduction of the experimental binding poses and providing confidence in the predicted interactions and binding affinities of apigenin-7-*O*-glucuronide.

Compound **1** fully complies with Ghose's rule and exhibits between one and three violations of Lipinski's, Veber's, and Muegge's rules. According to Ghose's rule, the refractive index of compound **1** (106.72) is normal, which could explain its membrane permeability, as indicated by the positive consensus Log P value (0.17). The high TPSA value (187.12) for this compound indicates low oral bioavailability, consistent with the low gastrointestinal absorption observed. Compound **1** is not expected to cross the blood-brain barrier (BBB), suggesting limited penetration into the central nervous system, an advantage for avoiding neurotoxicity. Compound **1** is also a substrate of P-glycoprotein, meaning its bioavailability can be further improved. Compound **1** does not inhibit any CYP enzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4), thus minimizing the risk of drug interactions via metabolic pathways. Compound **1** is water-soluble, has good membrane permeability and possesses properties that prevent drug interactions and CYP-related interference. This compound, which has a synthetic accessibility value of 5.28, is also easy to synthesize. However, it has low bioavailability due to the intestinal permeability barrier associated with low gastrointestinal absorption. These limitations in terms of drug properties do not rule out the therapeutic potential of compound **1**, but suggest the need for new strategies. Numerous techniques can improve the bioavailability of promising natural compounds by increasing their solubility, including particle size reduction,

complex formation, and conversion to prodrugs. Similarly, permeability can be improved by using an activator or encapsulating the compound in a lipid-based drug delivery system (34). In an *in vivo* study on rats, researchers found that apigenin-7-*O*-glucuronide had high oral bioavailability due to its stability in the gastrointestinal lumen and a significant intestinal first-pass effect. This demonstrates the potential of apigenin-7-*O*-glucuronide as a natural prodrug (35).

Conclusions

The CH_2Cl_2/MeOH extract of *P. febrifugum* leaves revealed the presence of compounds such as phenolics and flavonoids. Fractionation of this extract allowed the isolation of a flavone glycoside, apigenin-7-*O*-glucuronide, described here for the first time in this plant. The crude extract exhibited antiplasmodial activity *in vitro* against *P. falciparum* strains, while the isolated compound (apigenin-7-*O*-glucuronide) showed potential antiplasmodial relevance through *in silico* molecular docking studies. In addition, both the extract and the isolated compound demonstrated anti-inflammatory potential as evidenced by inhibition of egg albumin and BSA denaturation *in vitro*. Although the extract and apigenin-7-*O*-glucuronide demonstrated notable inhibitory effects in the egg albumin and BSA denaturation assays, these methods represent *in vitro* biochemical models based on protein stabilization and do not directly assess cellular inflammatory pathways. Therefore, the observed activities should be considered preliminary indicators of anti-inflammatory potential. Further studies employing cell-based assays and *in vivo* models are required to confirm the mechanisms and therapeutic relevance of these findings. Molecular modelling revealed that apigenin-7-*O*-glucuronide exhibited the strongest binding affinity and numerous interactions with BSA (4JK4), COX-1 (3NY8), *Pf*LDH (2X8L) and *Pf*DHODH (6GJG). Therefore, this compound could be a promising candidate for the development of BSA, COX-1, *Pf*LDH and *Pf*DHODH inhibitors. This plant could therefore be used as a natural source of anti-inflammatory and antiplasmodial agents.

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Plant Ethics: The plant material used in this study was derived from the wild plant *Psorospermum febrifugum*, which was collected under the supervision of a qualified botanist, Dr Fawa, from the Department of Biological Agriculture of the Faculty of Science, University of Ngaoundéré. The plant species was taxonomically identified at the National Herbarium of Cameroon. The herbarium is a publicly accessible collection, and deposited material can be accessed upon formal request. At the time and location of collection, *Psorospermum febrifugum* has not been classified as a protected or endangered species, and no specific licenses or permits were required under local or national regulations (Not applicable).

References

1. Medzhitov R. Inflammation 2010: new adventures of an old flame. Cell. 2010;140(6):771-776. CrossRef PubMed
2. Aidoo DB, Konja D, Henneh IT, et al. Protective effect of bergapten against human erythrocyte hemolysis and protein denaturation *in vitro*. Int J Inflamm. 2021;2021:1279359. CrossRef PubMed
3. Bouhlali EDT, Hmidani A, Bourkhis B, et al. Phenolic profile and anti-inflammatory activity of four Moroccan date (*Phoenix dactylifera* L.) seed varieties. Heliyon. 2020;6(2):e03436. CrossRef PubMed
4. Hasan AUH. Evaluation of *in vitro* and *in vivo* therapeutic efficacy of Ribes alpestre Decne in rheumatoid arthritis. Braz J Pharm Sci. 2019;55:e17832. CrossRef
5. Ginwala R, Bhavsar R, Chigbu DI, et al. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants. 2019;8(2):35. CrossRef PubMed
6. Silva IDR, Ramos UF, Cerqueira LE, et al. Antiplasmodial activity and phenolic composition of Brazilian *Salix humboldtiana* Willd. extract and fractions. J Med Plants Res. 2022;16(2):35-43. CrossRef
7. Crofford LJ. Use of NSAIDs in treating patients with arthritis. Arthritis Res Ther. 2013;15(Suppl 3)(suppl 3):S2. CrossRef PubMed
8. Boukhatem BS, Belhadj AE. *In silico* anti-inflammatory activity of lavender (*Lavandula officinalis*) essential oil bioactive compounds: molecular docking analysis of COX-1 and COX-2, and ADMET prediction. AIMS Allergy Immunol. 2023;7(2):132-153. CrossRef
9. World Health Organization. WHO malaria policy advisory group (MPAG) meeting report, 18–20 April 2023. World Health Organization; 2023.
10. Mbohou CN, Foko LPK, Nyabeyeu HN, et al. Malaria screening at the workplace in Cameroon. PLoS One. 2019;14(12):e0225219. CrossRef PubMed
11. Kotepui KU, Mahittikorn A, Mala W, et al. Total antioxidant status levels in malaria: a systematic review and *meta*-analysis. Malar J. 2024;23(1):198. CrossRef PubMed
12. Sarkar A, Basak S, Ghosh S, et al. Insights into the relation between oxidative stress and malaria: a mechanistic and therapeutic approach. React Oxyg Species (Apex). 2021;11:m26-m41. CrossRef
13. Akinnusi PA, Olubode SO, Adebesin AO, et al. Structure-based scoring of anthocyanins and molecular modeling of *Pf*LDH, *Pf*DHODH, and *Pf*DHFR reveal novel potential *P. falciparum* inhibitors. Inform Med Unlocked. 2023;38:101206. CrossRef
14. Rasool A, Bhat KM, Sheikh AA, et al. Medicinal plants: role, distribution and future. J Pharmacogn Phytochem. 2020;9(2):2111-2114.

15. Shi J, Cao H. Molecular structure-affinity relationship of dietary flavonoids for bovine serum albumin. Rev Bras Farmacogn. 2011;21(4):594-600. CrossRef
16. Ribeiro D, Freitas M, Tomé SM, et al. Flavonoids inhibit COX-1 and COX-2 enzymes and cytokine/chemokine production in human whole blood. Inflammation. 2015;38(2):858-870. CrossRef PubMed
17. Suresh PS, Kesarwani V, Kumari S, et al. Flavonoids from aerial parts of *Cissampelos pareira* L. as antimalarial agents: computational validation of ethnopharmacological relevance. S Afr J Bot. 2023;163:10-19. CrossRef
18. Rollando R, Maulada F, Afthoni MH, et al. Screening *Carica papaya* compounds as an antimalarial agent: *in silico* study. Trop J Nat Prod Res. 2023;7(5):2895-2903. CrossRef
19. Ajao AAN, Moteetee AN. *Psorospermum febrifugum* Spach (Hypericaceae): ethnomedicine, phytochemistry, and pharmacological activity of an underexplored plant. Sci Afr. 2023;22:e01934. CrossRef
20. Kumar G, Verma RK, Sable PD, et al. Medicinal values and phytochemical screening of fruits of Indian frankincense (*Boswellia serrata*). Roxb. Plants Second Metab; 2025:4.
21. Mirke NB, Shelke PS, Malavdkar PR, et al. *In vitro* protein denaturation inhibition assay of *Eucalyptus globulus* and Glycine max for potential anti-inflammatory activity. Innov Pharm Pharmacother. 2020;8(2):28. CrossRef
22. Derouich M, Bouhlali EDT, Hmidani A, et al. Assessment of total polyphenols, flavonoids and anti-inflammatory potential of three Apiaceae species grown in the Southeast of Morocco. Sci Afr. 2020;9:e00507. CrossRef
23. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science. 1976;193(4254):673-675. CrossRef PubMed
24. Smilkstein M, Sriwilaijaroen N, Kelly JX, et al. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. Antimicrob Agents Chemother. 2004;48(5):1803-1806. CrossRef PubMed
25. Lambros C, Vanderberg JP. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. J Parasitol. 1979;65(3):418-420. CrossRef PubMed
26. Chemical Computing Group ULC. Molecular Operating Environment (MOE). Chemical Computing Group ULC; 2014.
27. Javaid A, Ashfaq UA, Zafar Z, et al. Phytochemical analysis and antidiabetic potential of *Armoracia rusticana*: pharmacological and computational approach. Comb Chem High Throughput Screen. 2021;24(3):465-471. CrossRef PubMed
28. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7(1):42717. CrossRef PubMed
29. Feng JH, Lee HJ, Kim SB, et al. Antinociceptive effect of single components isolated from *Agrimonia pilosa* Ledeb. extract. Sci Pharm. 2019;87(3):18. CrossRef
30. Vélez LA, Delgado Y, Ferrer-Acosta Y, et al. Theoretical prediction of gastrointestinal absorption of phytochemicals. Int J Plant Biol. 2023;13(2):163-179. CrossRef
31. Lipinski CA, Lombardo F, Dominy BW, et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001;46(1-3):3-26. CrossRef PubMed
32. Veber DF, Johnson SR, Cheng HY, et al. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002;45(12):2615-2623. CrossRef PubMed
33. Muegge I, Heald SL, Brittelli D. Simple selection criteria for drug-like chemical matter. J Med Chem. 2001;44(12):1841-1846. CrossRef PubMed
34. Yu J, Wang P, Bei Z, et al. Recent Advances in oral drug delivery systems for BCS III drugs. Curr Issues Mol Biol. 2026;48(1):63. CrossRef PubMed
35. Seo SW, Choi SH, Hong JK, et al. Pharmacokinetics and extensive intestinal first-pass effects of apigenin and its active metabolite, apigenin-7-*O*-glucuronide, in rats. J Pharm Investig. 2024;54(4):467-481. CrossRef
36. Abongwa M, Samje M, Ayimele GA, et al. Filaricidal activity of *Daniellia oliveri* and *Psorospermum febrifugum* extracts. Parasit Vectors. 2021;14(1):305. CrossRef PubMed
37. Xue P, Zhang G, Zhang J, et al. Interaction of flavonoids with serum albumin: a review. Curr Protein Pept Sci. 2020;22(3):217-227. CrossRef PubMed
38. Rauf A, Khan R, Khan H, et al. Cytotoxic, antitumour-promoting and inhibition of protein denaturation effects of flavonoids, isolated from *Potentilla evestita* Th. Wolf. Nat Prod Res. 2015;29(18):1775-1778. CrossRef PubMed
39. Kumari DS, Satish PVV, Somaiah K, et al. Antimalarial activity of *Polyalthia longifolia* (False Ashoka) against chloroquine-sensitive *Plasmodium falciparum* 3D7 strain. World J Pharm Sci. 2016;4(6):495-501.
40. Mahfur M, Purwantini I, Wahyuono S, et al. *In vitro* antiplasmodial activities of the fractions of *Hyrtios reticulatus* sponge extract. J Appl Pharm Sci. 2022;12(9):114-120. CrossRef
41. Ringwald P, Basco LK. Comparison of *in vivo* and *in vitro* tests of resistance in patients treated with chloroquine in Yaoundé, Cameroon. Bull World Health Organ. 1999;77(1):34-43. PubMed
42. Abubakar A, Ahmad NS, Akanya HO, et al. Antiplasmodial activity of total alkaloids and flavonoids of stem bark extracts of *Enantia chlorantha* in mice. Comp Clin Pathol. 2020;29(4):873-881. CrossRef
43. Bankole AE, Adekunle AA, Sowemimo AA, et al. Phytochemical screening and *in vivo* antimalarial activity of extracts from three medicinal plants used in malaria treatment in Nigeria. Parasitol Res. 2016;115(1):299-305. CrossRef PubMed
44. Ijaz F, Ahmad N, Ahmad I, et al. Two new anti-plasmodial flavonoid glycosides from *Duranta repens*. J Enzyme Inhib Med Chem. 2010;25(6):773-778. CrossRef PubMed
45. Bi S, Wu J, Sun X, et al. Spectral and molecular docking studies on the interaction of three flavonoids with bovine serum albumin. J Biomol Struct Dyn. 2020;38(7):2197-2205. CrossRef PubMed
46. Sharef AY, Hamdi BA, Alrawi RA, et al. *Onopordum acanthium* L. extract attenuates pancreatic *β*-Cells and cardiac inflammation in streptozocin-induced diabetic rats. PLoS One. 2023;18(1):e0280464. CrossRef PubMed
47. Njiro BJ, Mutagonda RF, Chamani AT, et al. Molecular surveillance of chloroquine-resistant *Plasmodium falciparum* in sub-Saharan African countries after withdrawal of chloroquine for treatment of uncomplicated malaria: a systematic review. J Infect Public Health. 2022;15(5):550-557. CrossRef PubMed