

Research Article

Bioassay-Guided Fractionation of *Hunteria Umbellata* (K.Schum.) Hallier F. Root Reveals Selective Antiplasmodial Activity Against Chloroquine-Sensitive and Multidrug-Resistant *Plasmodium Falciparum*

Kyahar I. F.^{1*}, Ogaji J. I.²

^{1*}Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Bingham University Karu.

²Department of Pharmaceutics Microbiology and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of Jos.

Corresponding Author: Kyahar I. F

Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Bingham University Karu.

Email: kyaharfriday@yahoo.com

Received: 20.08.26, Revised: 15.09.26, Accepted: 08.10.26

ABSTRACT

Ethnopharmacological Relevance: *Hunteria umbellata* (K.Schum.) Hallier f. is used in West African traditional medicine for several ailments, including febrile conditions. However, evidence for antiplasmodial activity of its root and enrichment of active constituents by bioassay-guided fractionation is limited.

Aim of the Study: To investigate the antiplasmodial activity of *H. umbellata* root against chloroquine-sensitive 3D7 and multidrug-resistant Dd2 *Plasmodium falciparum* and assess activity enrichment by bioassay-guided fractionation.

Materials and Methods: Root material was sequentially extracted with solvents of increasing polarity. Extracts were screened for phytochemical constituents, and the most active extract was fractionated by vacuum liquid chromatography and further purification. Antiplasmodial activity against 3D7 and Dd2 was assessed using a SYBR Green I assay. Cytotoxicity was evaluated in Vero cells using the MTT assay. Selectivity and resistance indices were calculated.

Results: The dichloromethane extract showed the highest activity, with IC₅₀ values of 7.12 ± 0.02 and 8.68 ± 0.02 µg/mL against 3D7 and Dd2, respectively. Fraction F5 showed increased activity (IC₅₀ 3.50 ± 0.20 and 4.20 ± 0.20 µg/mL), while bioactive component B showed the highest activity (2.35 ± 0.02 and 2.80 ± 0.02 µg/mL). Bioactive Component B had a Vero-cell CC₅₀ of 132.80 ± 1.85 µg/mL, with selectivity indices of 56.51 and 47.43. Resistance indices ranged from 1.19 to 1.22.

Conclusion: Bioassay-guided fractionation enriched antiplasmodial activity in *H. umbellata* root. Bioactive component B retained activity against Dd2 and showed favourable in vitro selectivity, supporting further chemical characterization and mechanistic investigation. The findings are consistent with the documented traditional use of the species for febrile conditions but do not establish traditional antimalarial efficacy.

Keywords: *Hunteria Umbellate*, *Plasmodium Falciparum*, Antiplasmodial Activity, Bioassay-Guided Fractionation, Multidrug Resistance, Selectivity Index.

Highlights

- Root extract showed 7.12–8.68 µg/mL inhibitory activity against parasites.
- Fraction F5 improved potency from 7.12 to 3.50 µg/mL.
- Bioactive component B reached 2.35 µg/mL activity against the sensitive strain.
- Bioactive component B showed selectivity indices above 47 in mammalian cells.
- Resistance indices remained between 1.19 and 1.22 during fractionation.

INTRODUCTION

Malaria is still one of the most significant parasitic diseases globally and continues to be a major public health issue, especially in sub-Saharan Africa. The World Health Organization (WHO, 2024) reports that there were approximately 263 million cases of malaria and 597,000 deaths worldwide in 2023. *Plasmodium falciparum* is the main species responsible for severe cases and fatalities due to its high pathogenicity and its ability to develop resistance to antimalarial medications.

The rise and spread of resistance to antimalarial drugs pose one of the most serious challenges to global efforts aimed at controlling and eliminating malaria. The resistance to chloroquine and sulfadoxine-pyrimethamine has significantly contributed to past increases in malaria-related illness and death. Moreover, recent findings of partial artemisinin resistance and decreased susceptibility to artemisinin-based combination therapies have raised alarms about the long-term viability of current treatment approaches (Balikagala et al., 2021; Siqueira-Neto et al., 2023; Uwimana et al., 2020; WHO, 2024). The ongoing evolution of resistant parasite populations underscores the critical need for new antimalarial drugs with innovative chemical structures and mechanisms of action that can bypass existing resistance pathways.

Natural products have long been crucial in the search for antimalarial drugs. Notable antimalarial treatments like quinine and artemisinin have their roots in medicinal plants and are still considered some of the most effective natural-product-based therapies. These natural products continue to offer a wide range of chemical structures with distinct biological activities, making them essential sources for developing new antimalarial drugs (Atanasov et al., 2021; Newman & Cragg, 2020; Siqueira-Neto et al., 2023). One effective approach for discovering antiplasmodial lead compounds from medicinal plants is bioassay-guided fractionation, which helps pinpoint biological activity and isolate the active ingredients (Bekono et al., 2020; Bero et al., 2009; Tajuddeen & Van Heerden, 2019).

The plant chosen for this study, *Hunteria umbellata* (K.Schum.) Hallier f. is a medicinal plant documented in West African traditional medicine for the management of fever and other ailments. Specimens were gathered from wild populations in Moniya, Akinyele Local Government Area, Oyo State, Nigeria, during the period from May to October 2025. The plant material was botanically identified and authenticated at the Herbarium Unit of the Federal College of Forestry, Jos, Plateau State, Nigeria, and a voucher specimen (FHJ 205) was deposited in the herbarium.

The plant investigated in this study, *Hunteria umbellata* (K.Schum.) Hallier f. has a documented history of traditional medicinal use in West Africa. Historical and authoritative ethnomedicinal records indicate that the bark of *H. umbellata* is prepared as a bitter tonic and used for fever in Sierra Leone, while the roots

and bark have also been used traditionally for several other ailments in Nigeria and other West African countries (Dalziel, 1937; Oliver-Bever, 1986; Burkill, 1985; Neuwinger, 2000; Schmelzer & Gurib-Fakim, 2008). In addition, traditional use of *H. umbellata* fruit preparations for the management of fever has been reported in Nigeria (Igbe et al., 2009). These documented traditional uses, particularly the use of the plant in the management of fever, provide an ethnopharmacological basis for investigating its potential activity against malaria parasites. However, traditional use for fever should be distinguished from experimentally confirmed treatment of *Plasmodium falciparum* infection. The present study therefore evaluated the antiplasmodial activity of *H. umbellata* root extracts against chloroquine-sensitive 3D7 and multidrug-resistant Dd2 *P. falciparum* strains.

Phytochemical studies have revealed that *H. umbellata* is rich in a variety of secondary metabolites, such as alkaloids, flavonoids, triterpenoids, tannins, saponins, glycosides, and phenolic compounds. Notably, indole alkaloids and pentacyclic triterpenoids have gained significant interest due to their wide-ranging pharmacological effects and known antiparasitic properties. Research has shown that different extracts of *H. umbellata* exhibit antimicrobial, antioxidant, anti-inflammatory, antihypertensive, antidiabetic, analgesic, and antiplasmodial activities (Ali et al., 2019; Fadahunsi et al., 2021; Uka et al., 2025).

While there is growing evidence highlighting the medicinal significance of *H. umbellata*, most research to date has concentrated on crude extracts and initial biological screenings (Fadahunsi et al., 2021; Onyigeme-Okerenta et al., 2023). There is still a lack of information regarding its effectiveness against multidrug-resistant *P. falciparum* strains, its selectivity for mammalian cells, the bioassay-guided enrichment of active compounds, the mechanisms behind its antiplasmodial action, and the identification of the specific compounds that contribute to its observed effects. As a result, the potential of this species as an antimalarial lead has not been thoroughly explored.

Current strategies in antimalarial drug discovery are increasingly focused on finding parasite-selective compounds that can tackle multidrug resistance through innovative mechanisms (Burrows et al., 2017; Siqueira-Neto et al., 2023; Van Voorhis et al., 2016). The combination of bioassay-guided fractionation

with mechanistic assays and molecular docking has proven to be an effective method for discovering promising antimalarial lead compounds from medicinal plants. These integrated strategies help in pinpointing bioactivity, characterizing active constituents, and predicting interactions with established parasite targets involved in calcium homeostasis, folate metabolism, glycolysis, and heme detoxification pathways.

This study explored the antiplasmodial effects, cytotoxicity, selectivity, bioassay-guided fractionation, hemozoin inhibitory activity, phytochemical composition, and molecular interactions of *H. umbellata* root extracts on both chloroquine-sensitive (3D7) and multidrug-resistant (Dd2) strains of *Plasmodium falciparum*. Additionally, the research aimed to identify potential bioactive compounds linked to the observed effects and assess their viability as lead antimalarial agents against resistant malaria.

MATERIALS AND METHODS

Plant Material Collection and Identification

The plant chosen for this study, *Hunteria umbellata* (K.Schum.) Hallier f, is known for its traditional medicinal use in treating malaria and other febrile illnesses. Specimens were gathered from wild populations in Moniya, Akinyele Local Government Area, Oyo State, Nigeria, during the period from May to October 2025. The plant material was botanically identified and authenticated at the Herbarium Unit of the Federal College of Forestry, Jos, Plateau State, Nigeria, and a voucher specimen (FHJ 205) was deposited in the herbarium.

Preparation of Plant Extract

A serial exhaustive extraction was performed following the method outlined by Pandey, A. and Tripathi, S. (2014), utilizing solvents of increasing polarity: n-hexane, dichloromethane, ethyl acetate, n-butanol, and water. This approach was taken to ensure the extraction of phytochemicals with varying polarities.

One kilogram (1 kg) of powdered root material was macerated in 2.5 L of n-hexane for 24 hours with intermittent shaking. The mixture was then filtered through muslin cloth and subjected to vacuum filtration. The filtrates were concentrated under reduced pressure using a rotary evaporator set at 40 °C. The resulting extracts were dried, weighed, placed into sterile pre-weighed universal containers, and stored for future analysis. This procedure

was repeated sequentially with dichloromethane, ethyl acetate, n-butanol, and distilled water, after which the percentage extracted yield was calculated.

Qualitative Phytochemical Screening

Qualitative phytochemical screening was conducted using the standard methods outlined by Harborne, J. B. (1998) and Trease, G. E. and Evans, W. C. (2009). This screening aimed to identify the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds.

Determination of Total Phenolic Content (TPC)

The total phenolic content was assessed using the Folin–Ciocalteu method as described by Singleton, V. L. et al. (1999), with some minor adjustments. In brief, 0.5 mL of the extract solution (1 mg/mL in methanol) was combined with 2.5 mL of 10% Folin–Ciocalteu reagent and allowed to sit for 5 minutes at room temperature. After that, 2.0 mL of 7.5% sodium carbonate solution was added, and the mixture was incubated in the dark for 30 minutes.

The absorbance was recorded at 765 nm using a UV–Visible spectrophotometer, with a reagent blank as a reference. Gallic acid served as the calibration standard, and the total phenolic content was reported as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). All analyses were performed in triplicate.

Determination of Total Flavonoid Content (TFC)

To determine the total flavonoid content, we utilized the aluminum chloride colorimetric assay described by Chang, C. et al. (2002). Briefly, 0.5 mL of the extract solution (1 mg/mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride solution, 0.1 mL of potassium acetate solution, and 2.8 mL of distilled water, then incubated at room temperature for 30 minutes. After incubation, the absorbance was measured at 415 nm using a UV–Visible spectrophotometer. Quercetin was used as the calibration standard, and the flavonoid content was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract). All determinations were conducted in triplicate.

Determination of Total Alkaloid Content (TAC)

The total alkaloid content (TAC) was assessed using the alkaline precipitation gravimetric

method outlined by Harborne, J. B. (1998). In this process, 5 g of the dried extract was mixed with 200 mL of 10% acetic acid in ethanol and allowed to sit for 4 hours at room temperature, with occasional shaking. Afterward, the mixture was filtered and concentrated to about a quarter of its original volume using a water bath. A concentrated ammonium hydroxide solution was then added dropwise until the alkaloids completely precipitated. The resulting precipitate was allowed to settle and was collected through filtration with pre-weighed filter paper. The residue was washed with a dilute ammonium hydroxide solution, dried to a constant weight in an oven at 60°C, and then reweighed. The percentage of alkaloid content was calculated using the formula: % Alkaloid = (Weight of alkaloid residue / Weight of sample) × 100.

Reference Drugs

For reference drugs, chloroquine phosphate and artesunate were utilized as positive controls.

Parasite Strains, Source, and Culture Conditions

Laboratory-adapted *Plasmodium falciparum* reference strains 3D7 (chloroquine-sensitive) and Dd2 (multidrug-resistant) were sourced through collaboration with the National Institute for Pharmaceutical Research and Development (NIPRD) in Abuja, Nigeria. These well-characterized laboratory strains are recognized internationally as benchmark strains for antiparasmodial drug screening and resistance studies, owing to their reproducibility, defined drug sensitivity profiles, and appropriateness for standardized in vitro evaluations.

Parasites were grown in human O⁺ red blood cells using RPMI-1640 medium that was enhanced with 25 mM HEPES, 0.2% sodium bicarbonate, 0.5% Albumax II, and 50 µg/mL gentamicin. The cultures were kept at a hematocrit of 2% and a parasitemia of 2% at 37 °C, under a gas mixture consisting of 90% N₂, 5% CO₂, and 5% O₂ (Trager & Jensen, 1976). The growth of the parasites was tracked using Giemsa-stained thin blood smears.

Molecular Confirmation of Parasite Genotype

Genotyping of the *pfm-dr1* gene was carried out following established molecular techniques for identifying antimalarial resistance markers, as outlined by Duraisingh, M. T. et al. (2000). DNA from 200 µL of packed infected erythrocytes

was extracted using a commercial DNA extraction kit. A segment of the *pfm-dr1* gene, which includes codons 86 and 184, was PCR-amplified under the following conditions: initial denaturation at 95 °C for 3 minutes; 35 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 1 minute; followed by a final extension at 72 °C for 5 minutes. The size of the amplicons was confirmed through agarose gel electrophoresis. The products were then bidirectionally Sanger-sequenced, and the chromatograms were analyzed to identify the codons at positions 86 (AAT = N, TAT = Y) and 184 (TAT = Y, TTT = F). The strains corresponded to the expected genotypes: 3D7 (N86/Y184) and Dd2 (86Y/184F).

Preparation of Extract and Drug Solutions

The crude extract was dissolved in dimethyl sulfoxide at a concentration of 10 mg/mL and then diluted with culture medium to create a series of concentrations, ensuring that the final DMSO concentration in the assay wells was 0.3% (v/v), which does not affect parasite growth. Stock solutions of Chloroquine phosphate and Artesunate were prepared separately at a concentration of 1 mg/mL. Chloroquine phosphate was dissolved in sterile distilled water, while artesunate was first dissolved in dimethyl sulfoxide (DMSO) before being diluted with the culture medium. These solutions were then filter-sterilized using a 0.22 µm membrane filter and stored at -20 °C until needed. The validity of the assay was confirmed when the reference drugs yielded IC₅₀ values that fell within the accepted ranges for SYBR Green I antiparasmodial assays against the 3D7 and Dd2 strains of *Plasmodium falciparum*. The results showed that Chloroquine had IC₅₀ values of 0.02 µg/mL for the 3D7 strain and 0.85 µg/mL for the Dd2 strain, while Artesunate had values of 0.01 µg/mL for 3D7 and 0.03 µg/mL for Dd2. The drugs served as positive controls, and untreated infected erythrocytes were used as the negative control.

In Vitro Antiparasmodial Assay (Sybr Green I Fluorescence Method)

The in vitro antiparasmodial activity of various extracts, fractions, and isolated compounds was assessed using the SYBR Green I fluorescence assay, following the method outlined by Smilkstein et al. (2004) with some modifications. We maintained laboratory-adapted strains of *Plasmodium falciparum*, specifically the 3D7 (chloroquine-sensitive) and

Dd2 (multidrug-resistant) strains, in continuous culture as per the technique described by Trager and Jensen (1976).

The parasites were cultured in human O⁺ erythrocytes suspended in RPMI-1640 medium, which was supplemented with 25 mM HEPES, 0.2% sodium bicarbonate, 0.5% Albumax II, 50 µg/mL gentamicin, and hypoxanthine. These cultures were kept at 37°C in a gas mixture consisting of 90% N₂, 5% CO₂, and 5% O₂. To synchronize the parasites, we applied sorbitol treatment and adjusted the cultures to achieve 1% parasitaemia and 2% haematocrit before conducting the assay.

The erythrocytes were obtained from the Blood Bank/Blood Donation Laboratory of National Hospital, Abuja, from routinely screened donated blood, and were used only for an in-vitro laboratory assay. No participants were recruited or contacted by the research team, and no identifiable donor information was accessed.

We prepared stock solutions of the extracts, fractions, and isolated compounds in dimethyl sulfoxide (DMSO) and then serially diluted them in complete culture medium to create concentrations ranging from 0.39 to 100 µg/mL. The final concentration of DMSO in the assay wells was kept below 0.5% (v/v). Chloroquine and artesunate were used as positive controls, while untreated infected erythrocytes and DMSO-treated cultures served as negative controls.

Aliquots of synchronized parasite suspension were placed into 96-well microplates that contained the test samples and incubated at 37°C for 72 hours. After incubation, 100 µL of SYBR Green I lysis buffer was added to each well, and the plates were kept in the dark for 1 hour at room temperature. The fluorescence intensity was then measured using a microplate reader set to excitation and emission wavelengths of 485 and 535 nm, respectively. To calculate the percentage of parasite growth inhibition, the following formula was used:

$$\% \text{ Inhibition} = [(\text{Control} - \text{Treated}) / \text{Control}] \times 100$$

Dose-response curves were created using GraphPad Prism version 10.0, and IC₅₀ values were determined through nonlinear regression analysis. The validity of the assay was confirmed when chloroquine and artesunate yielded IC₅₀ values that fell within the accepted ranges for SYBR Green I antiplasmodial assays. Antiplasmodial activity was categorized based on established criteria: highly active (IC₅₀ < 10 µg/mL), moderately active (10–50 µg/mL),

weakly active (50–100 µg/mL), and inactive (>100 µg/mL). The resistance index (RI) was calculated as IC₅₀(Dd2)/IC₅₀(3D7), with RI values of ≤2 indicating retained activity against resistant parasites. All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation.

Cytotoxicity Assessment and Selectivity Index Determination

The cytotoxicity of the dichloromethane (DCM) root extract, along with its active fraction (F5) and isolated Component B, was assessed using the MTT colorimetric assay on Vero cells, which are derived from the African green monkey kidney. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum and 1% antibiotic solution and kept at 37°C in a humidified environment with 5% CO₂.

In brief, Vero cells were plated in 96-well microplates at a density of 1 × 10⁴ cells per well and allowed to adhere for 24 hours. After this period, the cells were treated with different concentrations of the test samples for 48 hours. Following the incubation, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for an additional 4 hours. The formazan crystals formed were then dissolved in dimethyl sulfoxide (DMSO), and the absorbance was measured at 570 nm using a microplate reader.

Cell viability was calculated in relation to untreated control cells, and CC₅₀ values were determined through nonlinear regression analysis using GraphPad Prism version 10.0. The Selectivity Index (SI) was calculated using the formula:

$$SI = CC_{50} / IC_{50}$$

where CC₅₀ indicates the concentration that leads to a 50% reduction in Vero cell viability, and IC₅₀ signifies the concentration that results in a 50% inhibition of parasite growth. SI values exceeding 10 were deemed indicative of favorable selectivity towards the parasite (Cos et al., 2006).

Bioassay-Guided Fractionation and Purification

The most potent dichloromethane (DCM) root extract of *Hunteria umbellata* (K.Schum.) Hallier f underwent bioassay-guided fractionation through vacuum liquid chromatography (VLC) on silica gel, following the method established by Coll and Bowden (1986) and adapted by Oluah et al. (2020). In summary, 28 g of the extract was applied to silica gel and fractionated using a gradient

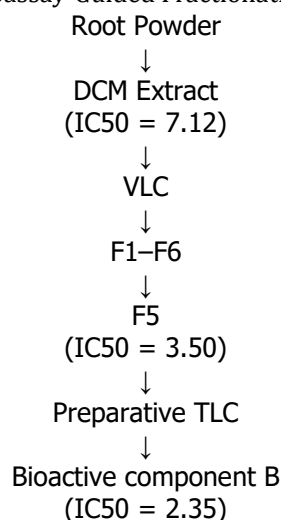
elution with solvent systems that increased in polarity, including n-hexane, hexane–dichloromethane, dichloromethane, dichloromethane–ethyl acetate, and ethyl acetate.

The fractions were monitored via thin-layer chromatography (TLC), and those with similar chromatographic profiles were combined to create six main fractions (F1–F6). These pooled fractions were then concentrated under reduced pressure and tested for antiplasmodial activity against chloroquine-sensitive (3D7) and

multidrug-resistant (Dd2) strains of *Plasmodium falciparum* using the SYBR Green I fluorescence assay.

The most effective fraction (F5) was further purified through repeated preparative TLC with hexane–ethyl acetate (80:20, v/v) as the mobile phase. The separated bands were collected, concentrated, and assessed for antiplasmodial activity. The resulting subfractions (Bioactive components A–C) were then subjected to biological evaluation and spectroscopic characterization.

Figure 1: Bioassay-Guided Fractionation Workflow



Statistical Analysis

In our study, experiments were conducted in triplicate, and the results are presented as mean ± standard deviation. One-way ANOVA along with Tukey's post-hoc test were used, which revealed significant decreases in IC₅₀ values throughout the purification process ($p < 0.05$).

RESULTS

Extraction Yield and Phytochemical Composition of *Hunteria Umbellata* Root Extracts

The extraction yield and phytochemical composition of *Hunteria umbellata* root extracts were analyzed. As shown in Tables 1, 2, and 3, the sequential extraction of the roots using solvents with increasing polarity resulted in

extracts that varied in both yield and phytochemical content. The extraction with dichloromethane (DCM) yielded the highest amount among the organic solvents at 8.65%, indicating a concentration of moderately polar compounds, while hexane produced a significantly lower yield of only 0.55%.

Qualitative screening of the phytochemicals revealed that the DCM extract was rich in alkaloids, flavonoids, terpenoids, and phenolic compounds, whereas the hexane extract was deficient in most of these metabolites. The diverse range of phytochemicals in the DCM extract suggests a greater likelihood of containing bioactive antiplasmodial compounds, which supports its selection for further bioassay-guided fractionation.

Tabel 1: Colour and Extracting Yield (%) of Root Extracts of *Hunteria Umbellata*

Plant Part	Weight of sample (g)	Extracting solvent (2.5 l)	Colour/Texture of extract	Weight of extract (g)	Yield (%)
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Root	1000	Hexane	Black	5.49	0.55
Root	1000	Dichloromethane	Dark brown	86.45	8.65
Root	1000	Ethyl acetate	Brown	70.45	7.05
Root	1000	Butanol	Pink	55.93	5.60
Root	1000	Water	Brown	35.54	3.55

Table 2. Phytochemical Composition of *Hunteria Umbellata* Root Extract

Parameter	Result
Total Phenolic Content	85.6 mg GAE/g
Total Alkaloid Content	42.3 mg/g
Total Flavonoid Content	12.8 mg QE/g

Table 3: Qualitative Phytochemical Constituents of *Hunteria Umbellata* Root Extract

Plant part	Extracting solvent	Terpenoids	Flavonoids	Alkaloids	Phenolics
Root	n-Hexane	-	-	-	-
Root	Dichloromethane	+	+	+	+
Root	Ethyl acetate	+	+	-	+
Root	n-Butanol	-	+	-	+
Root	Water	-	+	-	-

+ = Present, - = Absent

Antiplasmodial Activity of Crude Extracts

The antiplasmodial activities of the crude extracts against both chloroquine-sensitive (3D7) and multidrug-resistant (Dd2) strains of *P. falciparum* are summarized in Table 4. Among the extracts, the DCM extract stood out with the strongest activity, showing IC₅₀ values of 7.12 µg/mL for the 3D7 strain and 8.68 µg/mL for the Dd2 strain. The ethyl acetate extract displayed moderate activity, while the butanol and aqueous extracts were less effective, showing weak activity. Unfortunately, the hexane extract didn't show any activity against either of the parasite strains.

When looking at the DCM extract, fraction F5, and bioactive component B, they all had resistance indices of 1.22, 1.20, and 1.19 respectively (as shown in Table 5), which suggests that there was only a slight decrease in effectiveness against the resistant strain. A one-way ANOVA followed by Tukey's post-hoc test confirmed that the decreases in IC₅₀ values from the DCM extract to Fraction F5 and then to bioactive component B were statistically significant ($p < 0.05$). Based on the established criteria for antiplasmodial activity (IC₅₀ <10 µg/mL), the DCM extract was classified as highly active.

Table 4: Antiplasmodial Activity of *Hunteria Umbellata* Root Extracts Against Sensitive and Resistant *Plasmodium Falciparum* Strains

Type of Extracts	IC ₅₀ (µg/mL)-3D7, Mean ± SD	IC ₅₀ (µg/mL)-Dd2, Mean ± SD	Interpretation
Hexane	>100	>100	Inactive
DCM	7.12 ± 0.02	8.68 ± 0.02	Highly active
Ethyl acetate	12.00 ± 0.20	40.00 ± 0.20	Moderately active
Butanol	51.00 ± 0.20	60.00 ± 0.20	Weakly active
Water	>100	>100	Inactive
Chloroquine	0.02 ± 0.02	0.85 ± 0.02	Highly active
Artesunate	0.01 ± 0.02	0.03 ± 0.02	Highly active

Data are expressed as mean ± SD (n = 3). IC₅₀ values represent concentrations required to inhibit 50% parasite growth. Fractions with

IC₅₀ < 10 µg/mL were considered highly active; 10–50 µg/mL moderately active; 50–100 µg/mL weakly active; and >100 µg/mL inactive.

Table 5: Resistance Index (RI) of Extract, Fraction and Bioactive Component B

Sample	IC ₅₀ (µg/mL)-3D7, Mean ± SD	IC ₅₀ (µg/mL)-Dd2, Mean ± SD	RI Value Mean ± SD
DCM Extract	7.12 ± 0.02	8.68 ± 0.02	1.22 ± 0.20
Fraction, F5	3.50 ± 0.20	4.20 ± 0.20	1.20 ± 0.02
Bioactive component B	2.35 ± 0.02	2.80 ± 0.02	1.19 ± 0.02

Cytotoxicity and Selectivity of the DCM Extract, Fraction F5 & Bioactive Component B

Cytotoxicity

The cytotoxicity and selectivity of the dichloromethane (DCM) extract, Fraction F5, and bioactive component B were evaluated in relation to Vero cells. The results showed that all three samples had relatively low cytotoxicity, with CC₅₀ values recorded at 128.40 ± 1.25 µg/mL for the DCM extract, 145.60 ± 2.10 µg/mL for Fraction F5, and 132.80 ± 1.85 µg/mL for bioactive component B. Typically, plant-derived substances with CC₅₀ values exceeding 100 µg/mL are regarded as having low toxicity to mammalian cells. Thus, the CC₅₀ values observed indicate that these active samples have minimal harmful effects on normal mammalian cells, even at concentrations significantly higher than those needed to inhibit parasite growth. Notably, Fraction F5 had the highest CC₅₀ value at 145.60 µg/mL, suggesting that the fractionation process not only improved antiplasmodial activity but also slightly decreased cytotoxicity. This implies that some inactive or potentially harmful components may have been eliminated during purification.

In terms of selectivity, the selectivity index (SI) serves as a measure of the therapeutic window

for potential antimalarial agents. The DCM extract showed SI values of 18.03 against the 3D7 strain and 14.79 against the Dd2 strain. Fraction F5 demonstrated enhanced selectivity with SI values of 41.60 against 3D7 and 34.67 against Dd2. Component B exhibited the highest selectivity, with SI values of 56.51 against 3D7 and 47.43 against Dd2.

The steady rise in SI values from the crude extract to Fraction F5 and ultimately to Bioactive component B shows that we successfully enriched the parasite-selective compounds during the bioassay-guided fractionation process. SI values above 10 are typically seen as acceptable for identifying potential antimalarial leads, and all three samples demonstrated good selectivity. Notably, the very high SI values for Bioactive component B (over 45 against both parasite strains) suggest a strong preference for inhibiting the parasites while being less toxic to mammalian cells, making it a promising candidate for an antimalarial lead compound. Additionally, its ability to maintain high selectivity against the multidrug-resistant Dd2 strain further emphasizes the potential therapeutic significance of this isolated compound for treating resistant malaria infections.

Table 6. Cytotoxicity and Selectivity Indices of the Active Extract, Fraction and Isolated Component Against *Plasmodium Falciparum* Strains

Sample	IC ₅₀ (3D7) (µg/mL)	IC ₅₀ (Dd2) (µg/mL)	CC ₅₀ (Vero cells) (µg/mL)	SI (3D7)	SI (Dd2)
DCM Extract	7.12 ± 0.02	8.68 ± 0.02	128.40 ± 1.25	18.03	14.79
Fraction F5	3.50 ± 0.20	4.20 ± 0.20	145.60 ± 2.10	41.60	34.67
Bioactive component B	2.35 ± 0.02	2.80 ± 0.02	132.80 ± 1.85	56.51	47.43

Footnote: CC₅₀ = concentration causing 50% reduction in Vero cell viability; IC₅₀ = concentration causing 50% inhibition of parasite growth; SI = Selectivity Index (CC₅₀/IC₅₀).

Bioassay-Guided Fractionation of the Active Extract

Given its impressive activity, the DCM extract was chosen for bioassay-guided fractionation. Table 7 showcases the yield percentages of various VLC fractions and isolated components derived from the dichloromethane root extract of *Hunteria umbellata*. Vacuum liquid chromatography of 28.0 g of the dichloromethane (DCM) extract yielded six pooled fractions (F1–F6), with yields varying

from 9.64% to 27.21%. The standout was Fraction F5, which was the most plentiful, weighing in at 7.62 g and making up 27.21% of the total extract. Following closely were Fraction F3 at 18.21% and Fraction F4 at 15.54%. The notable yield of F5 indicates that a significant portion of the compounds in the DCM extract were concentrated in this fraction. What's particularly interesting is that F5 was also recognized as the most active fraction in the antiplasmodial assay. This suggests that the biological activity isn't just tied to a minor trace component but is actually found within a major phytochemical fraction of the extract. From a drug discovery standpoint, this is a promising finding, as it enhances the chances of isolating enough active compounds for further study and potential development.

After purifying F5, we ended up with three distinct components (A–C). The standout was Component B, which was collected in the largest quantity, 3.51 g, making up 46.06% of Fraction F5. In comparison, Components A and C contributed 20.20% and 19.42%,

respectively. The impressive yield of bioactive component B, along with its strong antiplasmodial activity, suggests it's the key bioactive player in Fraction F5.

The way the activity shifted from the crude DCM extract to Fraction F5 and then to Bioactive component B showed that we successfully enriched the antiplasmodial compounds through bioassay-guided methods. Plus, the significant amount of Bioactive component B indicates that this active metabolite is quite abundant in the plant material, which is a crucial factor for future efforts in isolation, structural analysis, and developing potential lead compounds.

Bioactive component B also made up 12.54% of the original DCM extract, highlighting the effective recovery of this bioactive component during the fractionation and purification process. Its relatively high abundance makes it an appealing candidate for an antimalarial lead compound, as we can obtain substantial quantities from the crude extract without losing much during purification.

Table 7. Yield (%) Of VLC Fractions and Isolated Components Obtained from the Dichloromethane Root Extract of *Hunteria Umbellata*.

Fraction/Component	Weight (g)	Yield (%)
DCM extract used for VLC	28.00	100.0
F1	3.20	11.43
F2	2.85	10.18
F3	5.10	18.21
F4	4.35	15.54
F5	7.62	27.21
F6	2.70	9.64
Bioactive component A	1.54	20.20*
Bioactive component B	3.51	46.06*
Bioactive component C	1.48	19.42*

*Percentage yield relative to Fraction F5 (7.62 g).

Table 8 outlines the in vitro antiplasmodial activity of VLC fractions derived from the dichloromethane (DCM) root extract of *Hunteria umbellata*, tested against both chloroquine-sensitive (3D7) and multidrug-resistant (Dd2) strains of *Plasmodium falciparum*. Among the six fractions analyzed, Fraction F5 showed the most significant antiplasmodial activity, with IC_{50} values of 3.50 ± 0.20 μ g/mL for the 3D7 strain and 4.20 ± 0.20 μ g/mL for the Dd2 strain, indicating strong efficacy against both types of parasites. When compared to the original DCM extract, Fraction F5 displayed improved potency, suggesting that the bioactive compounds were effectively concentrated through bioassay-guided

fractionation. Additionally, the low resistance index (RI = 1.20) suggests that this fraction maintained its effectiveness against the multidrug-resistant Dd2 strain, showing only a slight decrease in activity.

Fraction F4, on the other hand, exhibited weak antiplasmodial activity, with IC_{50} values of 51.00 ± 0.20 μ g/mL and 60.00 ± 0.25 μ g/mL against the 3D7 and Dd2 strains, respectively. In contrast, Fractions F1, F2, F3, and F6 were found to be inactive, with IC_{50} values exceeding 100 μ g/mL for both parasite strains. As anticipated, the reference antimalarial drugs chloroquine and artesunate demonstrated strong activity, while the negative control (DMSO) showed no inhibitory effect. These

results highlight Fraction F5 as the key active fraction contributing to the antiplasmodial effects of the DCM root extract, supporting its

further purification and characterization of the active components.

Table 8: In Vitro Antiplasmodial Activity of VLC Fractions Obtained from the Dichloromethane Root Extract of *Hunteria Umbellata* Against Chloroquine-Sensitive (3D7) and Multidrug-Resistant (Dd2) *Plasmodium Falciparum*

Pooled Fraction	IC ₅₀ (3D7) (µg/mL)	IC ₅₀ (Dd2) (µg/mL)	Interpretation
F1	>100	>100	Inactive
F2	>100	>100	Inactive
F3	>100	>100	Inactive
F4	51.00 ± 0.20	60.00 ± 0.25	Weakly active
F5	3.50 ± 0.20	4.20 ± 0.20	Highly active
F6	>100	>100	Inactive
Chloroquine	0.02 ± 0.02	0.85 ± 0.02	Highly active
Artesunate	0.01 ± 0.02	0.03 ± 0.02	Highly active
DMSO	>100	>100	Inactive

Purification and Antiplasmodial Activity of Bioactive Component:

The isolation of the bioactive component was achieved through repeated preparative TLC purification of Fraction F5, which resulted in three distinct components labeled A–C. Among these, Bioactive component B stood out, making up about 15.8% of Fraction F5 and proving to be the most active isolate.

Bioactive component B showed impressive IC₅₀ values of 2.35 µg/mL and 2.80 µg/mL against the 3D7 and Dd2 strains, respectively (see Table 8). Its resistance index of 1.19 suggests that it maintains effectiveness against resistant parasites.

As purification progressed, we noticed a steady decline in IC₅₀ values, dropping from 7.12 µg/mL for the DCM extract to 3.50 µg/mL for Fraction F5, and finally reaching 2.35 µg/mL for Bioactive component B. This nearly three-fold

increase in potency highlights the successful enrichment of antiplasmodial compounds through bioassay-guided fractionation, pinpointing the biological activity to a specific phytochemical component.

In summary, these findings suggest that the antiplasmodial properties of *H. umbellata* roots are concentrated in a particular bioactive fraction and its isolated constituent, which continues to exhibit strong activity against multidrug-resistant *P. falciparum* parasites. GC–MS analysis of Bioactive component B yielded tentative library matches to ursolic acid (47.84%; similarity index 94%) and oleanolic acid (42.14%; similarity index 92%). These assignments were based on spectral library matching and should therefore be regarded as tentative pending confirmation with authentic standards and/or complementary spectroscopic techniques.

Table 9: Antiplasmodial Activity of Bioactive Components of Fractions from Dichloromethane Root Extract of *Hunteria Umbellata* Against Sensitive and Resistant *Plasmodium Falciparum* Strains

Isolated Component	IC ₅₀ (µg/mL)-3D7 Mean ± SD	IC ₅₀ (µg/mL)-Dd2 Mean ± SD	Interpretation
Component A	12.00 ± 0.20	40.00 ± 0.20	Moderately active
Component B	2.35 ± 0.02	2.80 ± 0.02	Highly active
Component C	>100	>100	Inactive
Chloroquine	0.02 ± 0.02	0.85 ± 0.02	Highly active
Artesunate	0.01 ± 0.02	0.03 ± 0.02	Highly active
DMSO	>100	>100	Inactive

DISCUSSION

Malaria continues to be a major infectious disease globally, posing a significant public health challenge, especially in sub-Saharan Africa. The World Health Organization (WHO,

2024) reported that in 2023, there were around 263 million cases of malaria and nearly 597,000 deaths related to the disease, with *Plasmodium falciparum* being the primary cause of severe illness and fatalities. The rise of drug-resistant

parasite strains has heightened the urgency for discovering new antimalarial treatments (Balikagala et al., 2021; Siqueira-Neto et al., 2023; WHO, 2024). Medicinal plants are crucial sources of diverse compounds that could lead to new antimalarial discoveries (Atanasov et al., 2021; Newman & Cragg, 2020; Siqueira-Neto et al., 2023).

In our study, we found that the polarity of the solvent used for extraction had a significant impact on both the yield and the phytochemical profile. Among the organic solvents tested, dichloromethane yielded the highest amount and was rich in a variety of phytochemicals, such as alkaloids, flavonoids, terpenoids, and phenolic compounds. This aligns with findings from other studies on African medicinal plants, which indicate that medium-polarity solvents like dichloromethane and ethyl acetate are effective in extracting bioactive secondary metabolites linked to antiplasmodial properties (Ali et al., 2019; Bekono et al., 2020; Fadahunsi et al., 2021). The DCM extract showed a notably high total phenolic content of 85.6 mg GAE/g and an alkaloid content of 42.3 mg/g, indicating a significant presence of metabolites often associated with antimalarial effects.

The phytochemical makeup found in the DCM extract could help explain its strong antiplasmodial effects. Alkaloids are among the most significant classes of plant-based antimalarial agents, with well-known examples like quinine and various indole alkaloids sourced from the Apocynaceae family. Research has shown that alkaloids play a crucial role in combating malaria by disrupting the parasite's growth through mechanisms such as hindering heme detoxification, blocking nucleic acid synthesis, and interfering with vital metabolic functions (Ali et al., 2019; Bero et al., 2009; Tajuddeen & Van Heerden, 2019). Similarly, flavonoids and phenolic compounds have been found to have antiplasmodial properties by modulating antioxidants, inhibiting parasite enzymes, and working synergistically with other bioactive components (Atanasov et al., 2021; Tajuddeen & Van Heerden, 2019). The presence of these various phytochemical groups indicates that the observed activity in the crude extract may stem from both the individual effects and the combined actions of multiple metabolites.

The DCM extract showed the most potent antiplasmodial activity among all the extracts tested, effectively targeting both chloroquine-sensitive (3D7) and multidrug-resistant (Dd2) strains of *P. falciparum*, with IC₅₀ values of

7.12 and 8.68 µg/mL, respectively. Based on the classification by Cos et al. (2006), extracts with IC₅₀ values under 10 µg/mL are deemed highly active. The results from this study are quite promising when compared to various medicinal plant extracts that have been explored for antimalarial drug development. For instance, systematic reviews of African antimalarial plants reveal that many crude extracts have IC₅₀ values between 10 and 50 µg/mL against *P. falciparum*, while only a limited number show activities below 10 µg/mL (Bekono et al., 2020; Tajuddeen & Van Heerden, 2019). Thus, the effectiveness of the DCM extract reinforces the traditional medicinal use of *H. umbellata* and underscores its potential as a valuable source of antiplasmodial compounds.

One of the key findings from this study was the sustained activity against the multidrug-resistant Dd2 strain. The DCM extract, along with Fraction F5 and Component B, showed resistance indices of 1.22, 1.20, and 1.19, respectively. Resistance index values that are close to one indicate only a slight decrease in effectiveness against resistant parasites, which is highly sought after in the search for new antimalarial leads. The Dd2 strain has mutations linked to resistance against various standard antimalarial medications, including changes in transporter proteins like PfCRT and PfMDR1. Therefore, compounds that continue to be effective against Dd2 parasites may operate through mechanisms that are different from those impacted by traditional resistance pathways (Balikagala et al., 2021; Siqueira-Neto et al., 2023; WHO, 2024). The low resistance indices found in this study suggest limited cross-resistance and highlight the potential of *H. umbellata*-derived compounds in tackling resistant malaria infections.

The consistently low resistance indices (1.19–1.22) observed during purification suggest that the active components are probably not significantly influenced by the resistance mechanisms linked to the Dd2 phenotype. This is particularly noteworthy since many traditional antimalarial medications show significant drops in effectiveness against resistant strains. The sustained activity indicates that the bioactive component(s) might engage with different parasite targets or bypass known resistance-related transport pathways.

Bioassay-guided fractionation effectively pinpointed the antiplasmodial activity within Fraction F5. This fraction demonstrated nearly double the potency compared to the original

DCM extract, with IC₅₀ values of 3.50 and 4.20 µg/mL against the 3D7 and Dd2 strains, respectively. The progressive increase in biological activity after fractionation is commonly seen as a sign of successful enrichment of active components and the removal of inactive matrix elements during purification. Similar enhancements in antiplasmodial potency after chromatographic fractionation have been documented for various African medicinal plants studied through bioassay-guided methods, where the active fractions typically represent the bulk of the biological activity found in crude extracts (Bekono et al., 2020; Bero et al., 2009; Tajuddeen & Van Heerden, 2019).

Further purification led to the isolation of Component B, which exhibited the strongest antiplasmodial activity, showing IC₅₀ values of 2.35 and 2.80 µg/mL against the 3D7 and Dd2 parasite strains, respectively. This represents a significant three-fold increase in potency compared to the original extract, confirming that the antiplasmodial activity is concentrated in a specific bioactive compound or a closely related group of compounds. The effectiveness of Component B is comparable to various plant-derived antiplasmodial fractions and partially purified isolates noted in recent research, many of which report IC₅₀ values ranging from 3 to 10 µg/mL against *P. falciparum* strains (Bekono et al., 2020; Tajuddeen & Van Heerden, 2019). While direct comparisons should be made with caution due to variations in assay conditions and parasite strains, the potency of Component B positions it among the more promising antiplasmodial candidates derived from African medicinal plants. A key observation was that Component B made up about 12.5% of the original DCM extract, suggesting that the active metabolite is present in relatively high quantities within the plant material. This trait is beneficial for future drug development, as it allows for easier large-scale isolation and minimizes losses during purification.

The evaluation of mammalian-cell toxicity showed that the DCM extract, Fraction F5, and Component B had relatively low cytotoxicity towards Vero cells, with CC₅₀ values all exceeding 128 µg/mL. Generally, plant-derived extracts and fractions with CC₅₀ values above 100 µg/mL are considered to have low cytotoxicity and are seen as safe for initial drug-discovery studies (Cos et al., 2006). Therefore, the results suggest that the observed antiplasmodial activity is unlikely due to nonspecific toxicity. Notably, Fraction F5 had a

slightly higher CC₅₀ value compared to the parent extract, indicating that the fractionation process may have eliminated some potentially harmful constituents while concentrating the biologically active compounds.

The selectivity indices from this study further highlight the therapeutic potential of the active samples. Generally, selectivity indices above 10 are seen as a sign of favorable parasite selectivity and are often used as a standard for identifying promising antimalarial leads (Cos et al., 2006). The DCM extract showed SI values of 18.03 and 14.79 against the 3D7 and Dd2 parasites, respectively, while Fraction F5 demonstrated significantly better selectivity. Notably, bioactive component B achieved SI values of 56.51 and 47.43 against the 3D7 and Dd2 parasites, respectively. These figures surpass the minimum threshold by more than four times, indicating a strong preference for inhibiting parasites over causing toxicity to mammalian cells. Similar selectivity profiles have only been reported for a few highly promising plant-derived antiplasmodial leads found in recent natural-product screening efforts (Cos et al., 2006).

Selectivity plays a crucial role in the early stages of identifying antimalarial leads. While it's important for compounds to exhibit strong antiplasmodial activity, those that lack specificity for the parasite often encounter issues in later development due to toxicity to the host. The remarkably high selectivity index (SI) values for bioactive component B, which exceed 45, surpass the minimum threshold recommended for prioritizing antimalarial leads, indicating a significant therapeutic window.

The noticeable increase in selectivity from the crude extract to bioactive component B highlights the success of the bioassay-guided fractionation approach used in this research. Notably, the sustained high selectivity against the multidrug-resistant Dd2 strain suggests that the active component maintains its specificity for the parasite, even in the face of genetic adaptations associated with resistance. These traits are especially important given the rising challenge of antimalarial drug resistance and underscore the potential of *H. umbellata* as a promising source for new antimalarial lead compounds.

The current findings strongly indicate that the roots of *Hunteria umbellata* contain powerful and selective compounds that are effective against both chloroquine-sensitive and multidrug-resistant strains of *P. falciparum*. The combination of their high potency, low toxicity

to cells, favorable selectivity indices, and minimal cross-resistance underscores the potential of this medicinal plant as a valuable source of antimalarial compounds that deserve further purification, structural analysis, pharmacological testing, and in vivo studies.

CONCLUSION

This research shows that the root of *Hunteria umbellata* has significant and selective antiplasmodial effects against both chloroquine-sensitive (3D7) and multidrug-resistant (Dd2) strains of *Plasmodium falciparum*. Among the various extracts tested, the dichloromethane extract demonstrated the most potent activity and favorable selectivity for the parasites. Through bioassay-guided fractionation, we were able to enhance the antiplasmodial activity progressively, leading to the isolation of Component B, which exhibited high potency, low toxicity to mammalian cells, excellent selectivity indices, and sustained effectiveness against resistant parasites. The consistently low resistance indices noted during purification suggest that there is limited cross-resistance with existing antimalarial medications. Overall, these results support the traditional use of *H. umbellata* in treating malaria and highlight the plant as a promising source for developing new antimalarial compounds. Further research is needed to

explore the structural properties, mechanisms of action, and in vivo effectiveness of the active components.

Credit Authorship Statement

Friday I. Kyahar: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing review & editing, Visualization, and Project administration.

Joshua I. Ogaji: Methodology, Investigation, Resources, Supervision, Validation, Writing review & editing, and Project administration.

Both Authors: Both authors contributed to the interpretation of the results, critically reviewed the manuscript, approved the final version, and agreed to be accountable for the work.

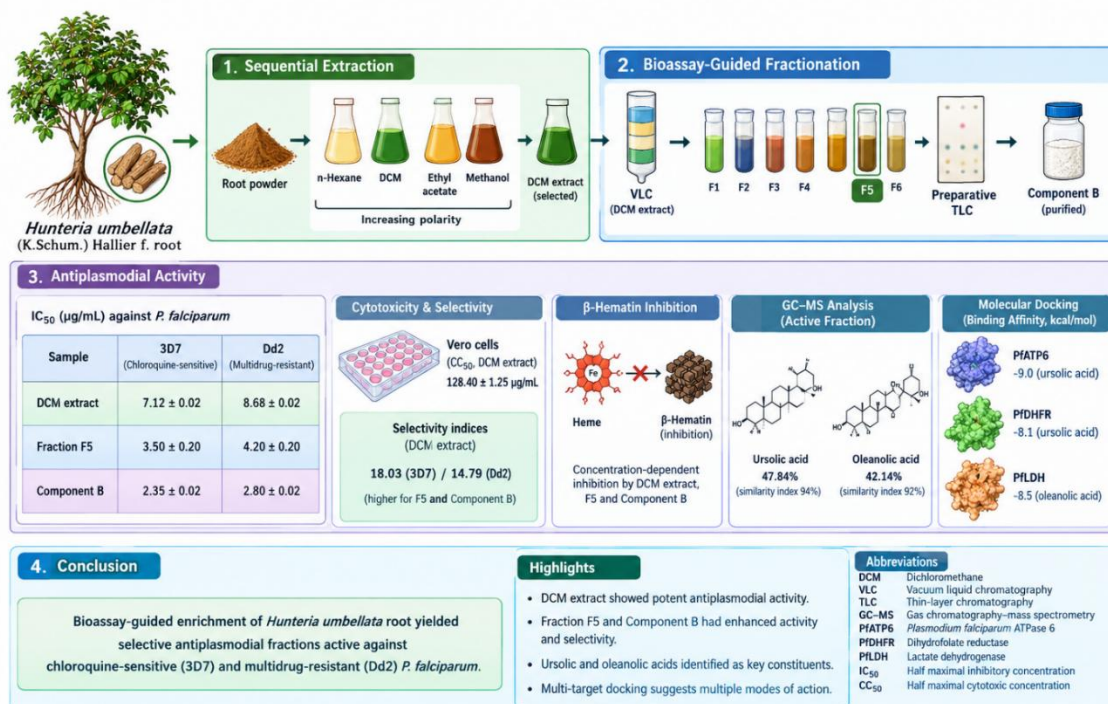
List Of Compounds Studied

The study investigated phytochemically enriched fractions and components obtained from the root of *Hunteria umbellata* (K.Schum.) Hallier f. by bioassay-guided fractionation. The principal bioactive material evaluated was designated Bioactive component B. Bioactive Component B... GC–MS library matching provided tentative matches to ursolic acid and oleanolic acid. This is why it is referred to as Bioactive component B throughout the manuscript rather than as a chemically identified compound.

List of Abbreviations

CC50	—	50%	cytotoxic	concentration
DCM	—	—	dichloromethane	
Dd2	—	multidrug-resistant	<i>Plasmodium falciparum</i>	strain
DMSO	—	—	dimethyl sulfoxide	
EtOAc	—	—	ethyl acetate	
F1–F6	—	vacuum liquid chromatography	fractions	1–6
FICI	—	fractional inhibitory concentration	index	
GAE	—	gallic acid	equivalent	
IC50	—	50% inhibitory concentration		
MTT	—	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide		
NIPRD	—	National Institute for Pharmaceutical Research and Development		
O+	—	O-positive blood group		
pfmdr1	—	<i>Plasmodium falciparum</i> multidrug resistance	1	gene
QE	—	quercetin	equivalent	
RI	—	resistance index		
RPMI	—	Roswell Park Memorial Institute	medium	
SD	—	standard deviation		
SI	—	selectivity index		
SYBR Green I	—	cyanine-based fluorescent nucleic acid stain used for parasite DNA quantification		
TLC	—	thin-layer chromatography		
Vero	—	African green monkey kidney cell line		
VLC	—	vacuum liquid chromatography		

A Graphical Abstract



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Characteristic FT-IR Absorption Bands of Ursolic Acid

Functional Group	Characteristic Peak (cm ⁻¹)	Assignment
O–H stretching	~3420	Hydroxyl group
C–H stretching	~2924	Aliphatic C–H
C=O stretching	~1689	Carboxylic acid carbonyl
C–H bending	~1375	Methyl bending
C–O stretching	~1049	Alcoholic C–O

Characteristic FT-IR Absorption Bands of Oleanolic Acid

Functional Group	Characteristic Peak (cm ⁻¹)	Assignment
O–H stretching	~3406	Hydroxyl group
C–H stretching	~2835–2864	Aliphatic C–H
C=O stretching	~1688	Carboxylic acid carbonyl
C=C stretching/bending	~1460	Olefinic/aliphatic vibration

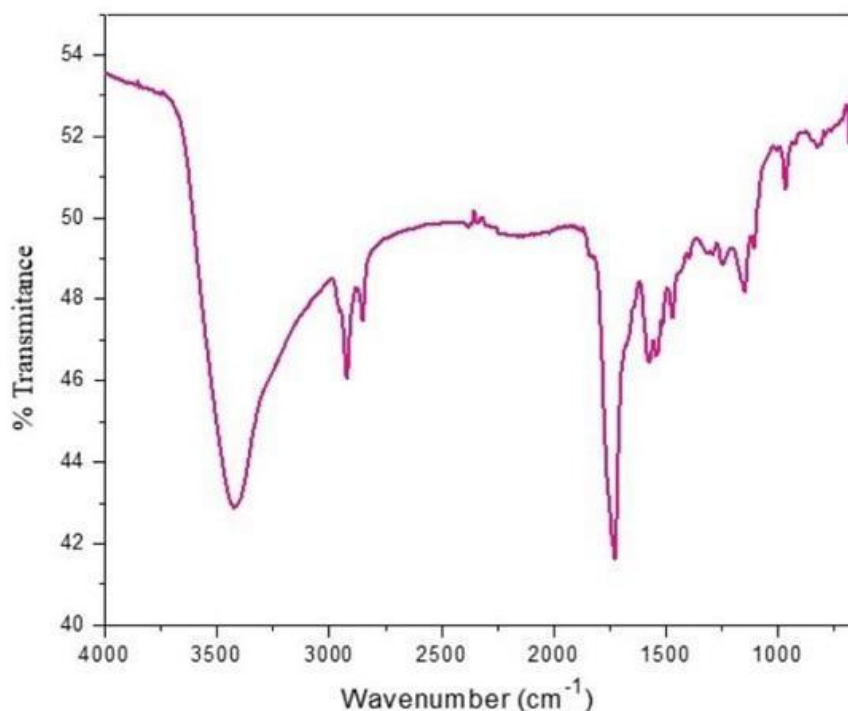


Figure 2: FT-IR Spectrum of DCM Root Extract of *Hunteria Umbellata* Showing Characteristic Absorption Bands of Pentacyclic Triterpenoids

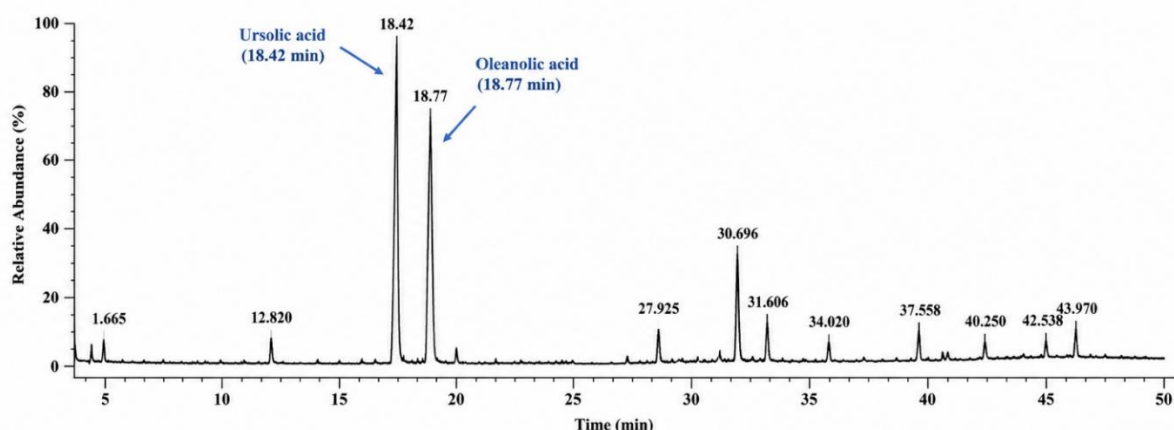


Figure 1: GC-MS chromatogram of DCM root extract of *H. umbellata*

Retention Time (min)	Compound Identified	Molecular Formula	Molecular Weight (g/mol)	Major Fragment Ions (m/z)	Similarity Index (%)
18.42	Ursolic acid	C ₃₀ H ₄₈ O ₃	456.7	203, 202, 320, 189	94
18.77	Oleanolic acid	C ₃₀ H ₄₈ O ₃	456.7	203, 202, 189, 320	92

Figure 3: GC-MS Chromatogram and GC-MS Library Search Identification Report of Compounds Detected in DCM Root Extract of *H. Umbellate*

Major Compounds Putatively Identified in the Most Active Fraction of DCM Root Extract of *Hunteria Umbellata* By GC-MS Analysis

Peak No.	RT (min)	Tentative Compound	Molecular Formula	Molecular Weight (g/mol)	Area (%)	Similarity Index (%)
1	18.42	Ursolic acid	C ₃₀ H ₄₈ O ₃	456.70	47.84	94
2	18.77	Oleanolic acid	C ₃₀ H ₄₈ O ₃	456.70	42.14	92

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3	Various	Minor unidentified constituents	ND	ND	10.02	<80
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ND = Not determined.