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Antiplasmodial-guided Isolation from Organic Extracts of the Root Bark of *Detarium microcarpum* Guill. & Perr.

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Abstract: In Niger, malaria remains a major public health problem, representing a leading cause of morbidity and mortality, particularly in children under five. Traditional medicine have significant importance, as it is often the first line of treatment due to its accessibility, cultural acceptability, and low cost. This study focused on the extraction, characterization, and antiplasmodial evaluation of bioactive compounds derived from the root bark of *Detarium microcarpum*. Successive extractions were performed using two solvents: methanol (100%) and a mixture of methanol and dichloromethane (1: 1). The obtained extracts were screened on chloroquine-sensitive (*Pf*3D7) and multidrug-resistant (*Pf*Dd2) *P. falciparum* strains using SyBr Green method. The most active dichloromethane-methanol extract (*Pf*3D7: IC_{50} = 11.98 ± 0.52 µg/mL and *Pf*Dd2: IC_{50} = 14.02 ± 0.17 µg/mL) was fractionated through liquid-liquid separation using n-hexane, ethyl acetate, and *n*-butanol. The ethyl acetate fraction yielded three isolated steroids (**1-3**) and two triterpenoids (**4-5**). Compound (**5**), identified as oleanolic acid, exhibited the highest activity (*Pf*3D7: IC_{50} = 4.82 ± 0.42 µg/mL and *Pf*Dd2: IC_{50} = 7.02 ± 0.27 µg/mL). Comprehensive analyses including phytochemical screening, chromatographic purification, spectral interpretation, and antiplasmodial activity assessment highlighted the chemical and pharmacological potential of the organic extracts. The overall results suggest that this plant is a promising source of molecules with therapeutic potential, especially for *in vitro* antiplasmodial activity. This work thus constitutes a significant contribution to the scientific valorization of local plant and opens up new possibilities for the development of phytomedicines derived from the African pharmacopoeia.

Introduction

Malaria remains a major health burden on the African continent, which accounted for approximately 94% of global cases and 95% of deaths related to the disease in 2022 (1). In Niger, this parasitic endemic constitutes a critical public health problem, representing one of the leading causes of consultations, hospitalizations, and mortality, particularly among children under five years of age (2). The persistence of high transmission rates, combined with challenges in achieving universal access to prevention measures and modern treatments, exacerbates population vulnerability.

Around half of the world's population is at risk of malaria, however most infections and deaths affect people in sub-Saharan Africa. In this region, P. falciparum is the species most commonly responsible for the high number of cases, due to the emergence of artemisinin-resistant pathogenic strains (3, 4). Artemisinin-resistant strains of P. falciparum have been detected across the world. This represents a significant challenge that compromises the current efforts to reduce or even eradicate malaria. In Niger, therapeutic failures with artemisinin-based combination therapy have been reported, highlighting the urgent need to tackle resistant parasites (5).

In this context, traditional medicine plays a central role in Niger's healthcare system, serving as the primary therapeutic recourse for a significant proportion of the population, especially in rural areas where access to formal health facilities remains limited (6). This reliance is supported by the rich flora of the Sahelian and Saharan regions, which represents a potential source of bioactive molecules. Indeed, the plant biodiversity of these ecosystems, used empirically for centuries, is the subject of scientific research aimed at validating its efficacy and isolating novel antimalarial active principles (7). Consequently, the ethnobotanical study of these medicinal

plants presents a highly relevant approach, both for documenting and preserving traditional knowledge and for contributing to the discovery of complementary therapies. Some plants used in the traditional pharmacopoeia of Niger and their derivatives (fractions and isolated compounds) have demonstrated antibacterial and antiplasmodial activities (8 – 10).

Detarium microcarpum, a tree found in African savannas, holds a significant place in traditional pharmacopoeias. Its various parts, particularly the root bark, are used to treat diverse ailments, including diarrhoea, infections, inflammations, and parasitic diseases. Several studies have confirmed its antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities, thereby reinforcing its pharmacological potential (11–13). However, studies on the antiparasitic activities of plants from Niger and their derivatives remain limited, which limits the availability of scientific data on their antiparasitic activity, particularly their anti-plasmodial potential. A few studies indicate anthelmintic potential of *D. microcarpum*. This disparity raises concerns since parasitic diseases remain a major public health problem in Africa and drug resistance is becoming increasingly severe.

Considering its traditional use against parasitic ailments and the presence of relevant bioactive metabolites (14), *D. microcarpum* represents a promising candidate for the search for novel antiparasitic agents (15). Few studies have been conducted on the antimalarial activity of extracts obtained from the organs of *D. microcarpum*. An antiplasmodial study was conducted on the hexane-defatted ethanolic extract obtained from the trunk bark of *D. microcarpum*. The tests were conducted on the chloroquine-resistant W2 strain of *Plasmodium falciparum*, and the 50% inhibitory concentration was 95.0 µg/mL (16). The results showed that this extract exhibits very low activity against strains of *P. falciparum* (16). Previous studies on this species were not focused on the root bark, which is an organ widely used in traditional medicine. There are very few, or no, studies that have investigated the isolation of compounds from *D. microcarpum* guided by their antiplasmodial activity. The objective of this research is to assess the anti-plasmodial properties of the root bark of *D. microcarpum* thereby contributing to the scientific validation of its traditional use.

Materials and Methods

Plant Material

Root bark of *D. microcarpum* Guill. & Perr. was freshly harvested in March 2023 in the Dosso region of Niger. Botanical identification of the material was performed by comparison with an authentic voucher specimen (reference number 756) deposited in the herbarium of Garba Mounkaila Laboratory of the Department of Biology at Faculty of Sciences and Techniques, Abdou Moumouni University. The samples were shade-dried at ambient temperature (27 ± 2 °C), protected from direct sunlight. After complete drying, the plant material was ground

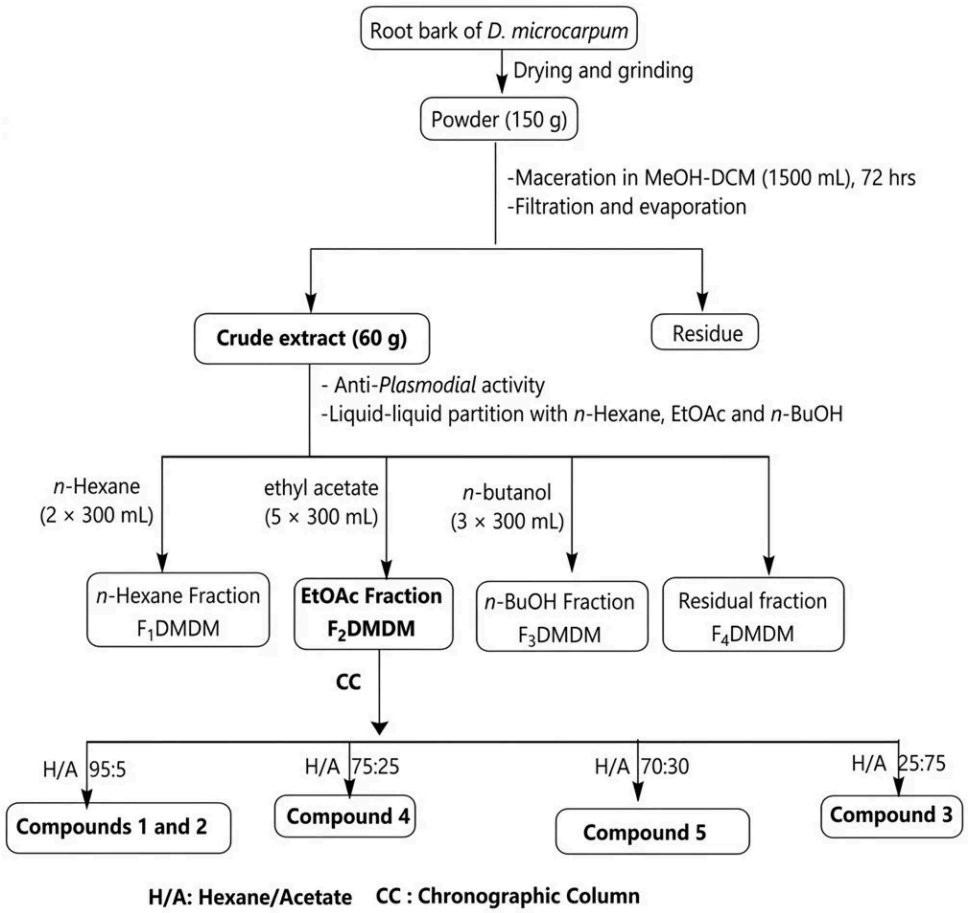


Figure 1. Fractionation procedure of the crude extract from the root bark of *D. microcarpum*.

Table 1. Evaluation scale for the antiplasmodial activity of extracts, fractions, and isolated compounds based on IC_{50} values.

Pure compounds	Extracts/Fractions	Activity
IC50 < 10 mg/mL	IC50 < 10 mg/mL	Excellent
10 mg/mL ≤ IC50 < 20 mg/mL	10 mg/mL ≤ IC50 < 50 mg/mL	Strong
20 mg/mL ≤ IC50 < 50 mg/mL	50 mg/mL ≤ IC50 < 100 mg/mL	Moderate
IC50 ≥ 50 mg/mL	IC50 ≥ 100 mg/mL	Inactive

using a mechanical grinder to obtain a homogeneous powder (17, 18).

Extraction, Fractionation, and Isolation

Root bark powder was subjected to maceration extraction using methanol (MeOH) and a dichloromethane-methanol (1:1, v/v) mixture as solvents. A quantity of 150 g of plant powder was immersed in each solvent (1500 mL) and intermittently agitated for 72 h at ambient temperature (**Figure 1**). Following filtration, the macerates were concentrated under reduced pressure using a rotary evaporator (19). The two crude extracts obtained were screened for antiplasmodial activity, and the most active extract was selected for further fractionation. 60 g of the crude extract prepared with dichloromethane-methanol (1:1, v/v) was subjected to liquid-liquid fractionation using *n*-hexane (2 × 300 mL), ethyl acetate (5 × 300 mL), and *n*-butanol (3 × 300 mL). This led to four fractions: the hexane fraction (F_1DMDM = 8.5 g), the ethyl acetate fraction (F_2DMDM = 34.8 g), the butanol fraction (F_3DMDM = 11.4 g), and the residual aqueous fraction (F_4DMDM = 5.3 g).

The ethyl acetate fraction (30 g) was purified using column chromatography with silica gel 60 (Merck, Darmstadt, Germany) as the stationary phase. The elution was carried out using a progressive polarity gradient with a mixture of n-hexane/ethyl acetate (100:0; 90:10; 80:20; 75:25; 50:50; 0:100). Forty-six subfractions of 100 mL each were collected. These were grouped based on their thin-layer chromatography (TLC) profiles. Fractions that exhibited identical or similar spots on TLC were grouped together. The spots corresponding to the compounds on the TLC plates were observed under UV light at 254 and 365 nm, then visualized by spraying with a sulfuric acid (H_2SO_4) solution followed by heating to 100 °C for 5 min. The purity of the isolated compounds was verified by 1D TLC, 2D TLC, and double migration. The pure compounds were subjected to NMR analysis. The NMR spectra (^{13}C, ^{1}H, HMBC, HSQC, DEPT 135) were analyzed using MestRelab software (Mnova version 14.3) to identify the characteristic peaks of carbon and proton nuclei. The spectra of the isolated compounds are presented in the supplementary materials.

Phytochemical Screening

A preliminary phytochemical screening of the extracts and fractions was performed using standard methods described in the literature (19, 20). The following chemical classes were identified: alkaloids, flavonoids, tannins, saponins, triterpenes/sterols, anthraquinones, and coumarins.

Evaluation of Antiparasitic Activity

The antiparasitic activity of the extracts and fractions was tested against two strains of *P. falciparum,* a multidrug-resistant strain (*PfDd2*) and a chloroquine-sensitive strain (*Pf3D7*). The assays were conducted according to standard protocols for *in vitro* parasite growth inhibition, enabling the determination of IC_{50} values. The interpretation of IC_{50} values was based on the scale established in published literature (21) (**Table 1**).

Plasmodium falciparum Culture Protocol

The culture technique used is that described by Trager and Jensen (1976) (22). The multidrug-resistant *PfDd2* and chloroquine-sensitive *Pf*3D7 strains of *P. falciparum* were cultivated in fresh human O-positive red blood cells at 4% hematocrit. Cultures were maintained in complete RPMI medium [500 mL RPMI 1640 (Gibco, UK) supplemented with 25 mM HEPES (Gibco, UK), 0.50% Albumax I (Gibco, USA), 1X hypoxanthine (Gibco, USA), and 20 µg/mL gentamicin (Gibco, China)] and incubated at 37 °C in a humidified incubator with a gas mixture of 92% N_2, 5% CO_2, and 3% O_2. The medium was replaced daily with fresh complete RPMI medium to facilitate parasite growth in culture. Thin blood smears were prepared, stained with Giemsa, and observed under a light microscope with oil immersion using a 100X objective to monitor all stages of the cell cycle and assess parasitemia.

Culture Synchronization

Prior to each antiplasmodial activity assay, parasite cultures consisting predominantly of ring stages (>80%) were synchronized to the same stage (ring stage) by treatment with 5% (w/v) sorbitol for 10 min, according to the protocol of Lambros and Vanderberg (1979) (23). Using synchronized cultures at the same stage, compared to mixed-stage cultures, allows for the evaluation of the effect of extracts, fractions, and isolated compounds across all three developmental phases (rings, trophozoites, and schizonts) over the 48-h life cycle of *P. falciparum*.

Preparation of Stock and Reference Solutions (Artemisinin, Chloroquine)

Stock and reference solutions were prepared in 10% DMSO at concentrations of 100 mg/mL and 100 µM, respectively, for the extracts and the artemisinin and chloroquine references. For this, 100 mg of extract were dissolved in 100 µL of dimethyl sulfoxide (DMSO). Following dissolution, the volume was adjusted to 1 mL with culture medium to obtain a 100 mg/mL stock solution. The resulting solution was used for the antiplasmodial activity assays.

Preparation of Intermediate Concentrations for Extracts and Positive Controls (Artemisinin, Chloroquine)

Intermediate concentrations of each sample were achieved by adding 10 µL of the extract stock solution (100 mg/mL)

Table 2. Extract yields.

Extract	Obtained mass (g)	Yield (%)
Methanolic	52 g	34.6%
Dichloromethane–methanol (1:1)	63 g	42%

and 20 µL of each positive control stock solution (100 µM) to 190 µL and 180 µL of incomplete RPMI-1640 medium, respectively, in a 96-well microplate. This was followed by a 5-fold geometric dilution series. The intermediate concentrations ranged from 8 to 5000 µg/mL for the extracts and fractions, and from 0.016 to 10 µM for the positive controls.

In Vitro Plasmodium falciparum Growth Inhibition Assay Using SYBR Green Fluorescence

The antiplasmodial activity of the extracts, fractions, isolated compounds, and positive controls was assessed in flat-bottom 96-well microplates using the SYBR Green I fluorescence-based method (24). The principle of this assay relies on the ability of SYBR Green I to produce strong fluorescence only in the presence of DNA, making it suitable for evaluating cellular proliferation. The absence of a cell nucleus in human red blood cells, within which the parasite proliferates, allows for specific monitoring of plasmodial growth using SYBR Green I.

Experimentally, 90 µL of a synchronized ring-stage parasite suspension (2% parasitemia, 1% hematocrit) was incubated with 10 µL of serially diluted test solutions at various concentrations of the extracts, fractions, isolated compounds, artemisinin, and chloroquine. The plates were then incubated at 37 °C for 72 h in a humidified incubator with a gas mixture of 92% N_2, 5% CO_2, and 3% O_2. The final concentrations in the test plates ranged from 0.8 to 500 µg/mL (with DMSO < 1%) for the plant extracts and from 0.0016 to 1 µM (with DMSO at 0.1%) for the positive controls, in a final volume of 100 µL. All experiments were performed in duplicate.

After a 72-h incubation period, 100 µL of SYBR Green I lysis buffer [composed of 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 (0.012% w/v saponin), and 28.8 µL of Triton X-100 (0.08% v/v)] was carefully added to each well. The plate was then incubated at 37 °C for 1 h in the dark.

Following this incubation, fluorescence was measured using an Infinite M200 (Tecan) microplate reader at excitation and emission wavelengths of 485 nm and 538 nm, respectively. The fluorescence values obtained were used to calculate % inhibition using Microsoft Excel software. The half-maximal inhibitory concentrations (IC_{50}) were determined from the dose-response curves generated by plotting the logarithm of the concentration against the % of inhibition, using GraphPad Prism 9 software.

Results and Discussion

Extract Yields

Maceration extraction of *D. microcarpum* root bark yielded two principal extracts. The obtained yields are presented in **Table 2**.

The dichloromethane-methanol extract exhibited the highest yield (42%), followed by the methanolic extract (34.6%). The relatively high yields observed for both extracts indicate a high content of extractable metabolites in the root bark of *D. microcarpum*. Polar and semi-polar solvents, such as methanol and DCM-MeOH, are recognized for their ability to efficiently extract a wide variety of bioactive compounds, including polyphenols, saponins, flavonoids, and triterpenes (19, 25).

Previous studies on *D. microcarpum* have reported similar yields using polar solvents. For instance, Constantin *et al*. (2015) demonstrated that methanolic extracts from roots and leaves provided the best yields compared to non-polar solvents (26) and Hama *et al*. (2025) reported a yield of 31.38% for methanolic extracts of *D. microcarpum* root bark (15). These findings are in line with the data from the present study, suggesting that root bark is particularly rich in extractable compounds. This provides scientific support for its traditional use in pharmacopoeia.

The higher yield of the dichloromethane-methanol (1:1) extract can be explained by:

1. The complementary polarity of the two solvents, enabling the simultaneous extraction of slightly non-polar compounds (sterols, terpenes, flavonoid aglycones) and polar compounds (saponins, condensed tannins).
2. The chemical nature of the plant material, which is rich in diverse secondary metabolites characteristic of species within the *D. microcarpum* genus (27, 28).

Thus, the DCM-MeOH extract optimizes the extraction of bioactive compounds potentially responsible for

Table 3. Phytochemical profile of *Detarium microcarpum* extracts.

Chemical family	MeOH	MeOH–DCM	n-Hexane	Ethyl acetate	n-Butanol
Alkaloids	–	–	–	–	–
Flavonoids	+	+	–	+	–
Tannins	+	+	–	+	+
Triterpenes	–	+	+	+	–
Sterols	–	+	+	+	–
Anthraquinones	–	+	+	–	–
Coumarins	–	–	–	+	–

Note: (–) = absence and (+) = presence

antiparasitic activities.

Phytochemical Screening

Phytochemical screening of the extracts and fractions derived from *D. microcarpum* root bark revealed the presence of several classes of secondary metabolites. Flavonoids and tannins were predominantly present in the polar and semi-polar extracts, whereas triterpenes, sterols, and anthraquinones were primarily found in the less polar fractions (**Table 3**).

The results demonstrate that the root bark of *D. microcarpum* contains a notable diversity of secondary metabolites, which is in line with literature data on this species (14, 25, 27).

There were no positive results for alkaloids in any extracts. This finding is consistent with several previous studies indicating that *D. microcarpum* is poor in alkaloids, particularly in its woody organs (28). This absence is significant, as alkaloids are often responsible for potent antiparasitic activities in many plant species (29).

Flavonoids were detected in the methanolic, MeOH-DCM, and ethyl acetate fraction extracts, but were absent from the hexane and butanol fractions. On the other hand, tannins, were widely distributed across almost all fractions except for the hexane fraction. This distribution aligns with the high polarity of tannins and polyphenols (20) and the fact that flavonoid aglycones readily partition into semi-polar solvents (19). Flavonoids and tannins are well-documented for their antiparasitic activities against *P. falciparum*, notably through mechanisms such as the inhibition of heme polymerization and the induction of oxidative stress (30).

Triterpenes and sterols were predominantly present in the hexane and ethyl acetate fractions, as well as in the MeOH-DCM extract. This distribution reflects their lipophilic nature, accounting for their insolubility in the pure methanolic extract (high polarity). Pentacyclic triterpenes (such as betulinic or oleanolic acid) are recognized for their antiparasitic properties (31), suggesting they could contribute to the activity observed in the semi-polar fractions.

Anthraquinones were detected in the MeOH-DCM extract and the hexane fraction, but were absent from the more polar fractions. Anthraquinones are metabolites with well-documented antimalarial properties, notably through the generation of free radicals toxic to the parasite (32). Their presence in the less polar fractions is consistent with their hydrophobic structure.

The presence of coumarins exclusively in the ethyl acetate fraction is notable. Coumarins are known for their antiparasitic activity, particularly via disruption of parasitic DNA synthesis (33). This could account for a portion of the activity likely associated with this fraction. The phytochemical screening clearly demonstrates that *D. microcarpum* contains a diverse array of bioactive molecules that may explain its antiparasitic activity. The ethyl acetate fraction stands out as the most diversified and richest in bioactive metabolites, which may correlate with strong antiplasmodial activity.

Isolation of Specialized Metabolites from *D. Microcarpum* Root Bark

Purification of the ethyl acetate fraction led to the isolation and characterization of five compounds (1-5) (**Figure 2**). The structures of the isolated compounds were determined based on their spectroscopic data (NMR and MS) and by comparison with those of similar compounds described in the literature. These compounds were identified as stigmasterol (**1**); spinasterol (**2**); spinasterol 3-O-*β*-D-glucopyranoside (**3**); oleanonic acid (**4**); and oleanolic acid (**5**), (see Supplementary Information **Figure S1** to **Figure S12** and **Table S1** to **Table S3**).

Identification of the Active Compound 5

Compound (**5**) precipitated as a white powder from the hexane/ethyl acetate (70:30, v/v) mixture and is soluble in chloroform. It gave a positive Liebermann–Burchard test, characteristic of triterpenes, producing a violet-red

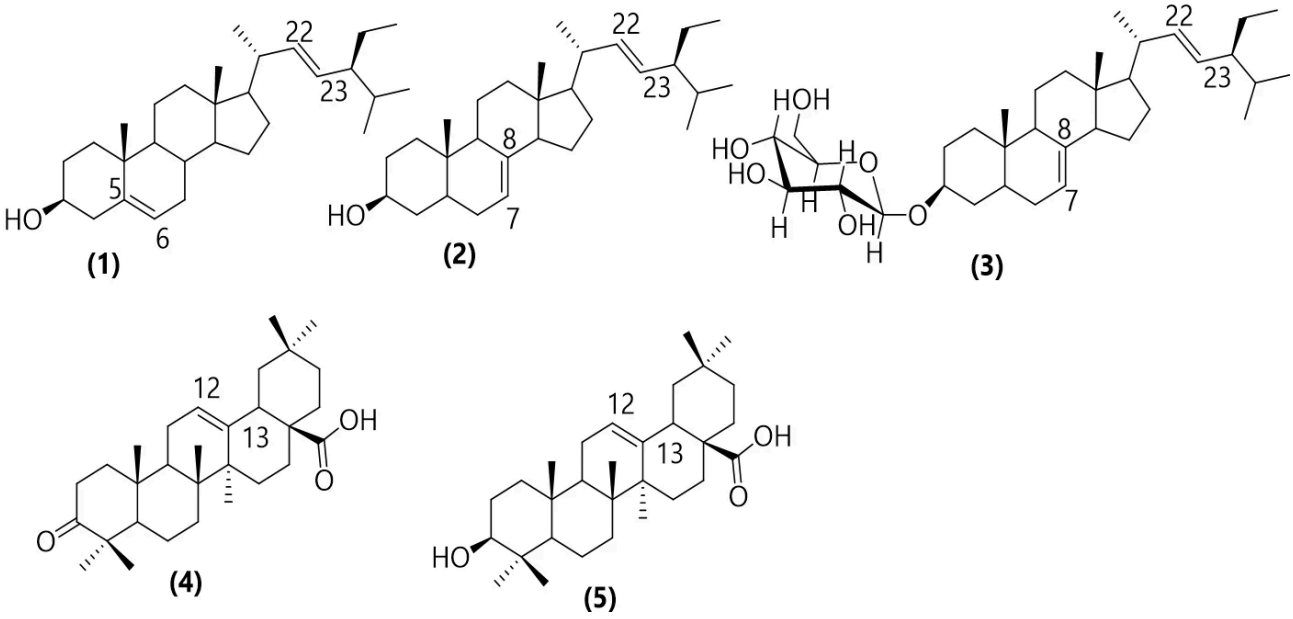


Figure 2. Chemical structures of isolated compounds 1–5.

coloration.

Its ^{1}H NMR spectrum (**Figure S11, Table 4**) displayed signals largely similar to those of compound (4). The major observed difference was the presence of a signal at δ_H = 3.42 ppm (dd, *J* = 4.3, 11.6 Hz) attributable to the oxymethine proton H-3. This suggests the reduction of the carbonyl ketone group at C-3 to an alcohol (CH-OH).

The ^{13}C NMR spectrum (**Figure S12, Table 4**) showed signals for 30 carbon atoms, with chemical shift values almost identical and similar with those of compound (4). Notably, the signal for the ketone carbonyl at δ_C = 217.5

Table 4.1H NMR (500 MHz) and 13C NMR (125 MHz) data for compound (**5**) in C_5D_5N compared with literature data in DMSO-d6 (500 MHz) (35).

Pos.	DMDM5		Litterature	
	δC (ppm)	δH (m, J en Hz)	δC (ppm)	δH (m, J en Hz)
1	38.7	0.94 (m)	38.4	0.98 (m) ; 1.63 (m)
2	27.8	1.62 (m) ; 1.57 (m)	27.2	1.61 (m) ; 1.56 (m)
3	77.8	3.42 (dd, 4.3, 11.6)	79.0	3.24 (dd, 4.3 ; 11.6)
4	39.2	-	38.8	-
5	55.6	0.84 (s)	55.2	0.74 (s)
6	18.5	1.60 (m) ; 1.42 (m)	18.3	1.57 (m) ; 1.39 (m;)
7	33.1	1.33 (m)	33.1	1.24 (m)
8	39.5	1.65 (m)	39.3	1.60 (m)
9	47.9	1.55 (m)	47.6	1.53 (m)
10	37.1	-	37.1	-
11	23.4	2.18 (m) ; 1.94 (m)	22.9	2.00 (m) ; 1.63 (m)
12	122.3	5.49 (t, 3.0)	122.7	5.28 (t, 3.5)
13	144.6	-	143.6	-
14	41.9	-	41.6	-
15	28.1	1.17 (m)	27.7	1.08 (m) ; 1.72 (m)
16	23.5	1.92 (m) ; 2.23 (m)	23.4	1.90 (m) ; 1.60 (m)
17	46.2	-	46.5	-
18	41.7	3.28 (dd, 4.5, 13.8)	41.0	2.83 (dd, 4.5, 13.8)
19	46.4	1.69 (m) ; 1.33 (m)	45.9	1.63 (m) ; 1.17 (m)
20	30.7	-	30.7	
21	33.9	1.33 (m) ; 1.51 (m)	33.8	1.24 (m) ; 1.34 (m)
22	33.0	1.79 (m) ; 2.05 (m)	32.4	1.78 (m)
23	28.5	1.23 (s)	28.1	0.98 (s)
24	16.3	0.80 (s)	15.6	0.77 (s)
25	15.3	0.88 (s)	15.3	0.91 (s)
26	17.2	1.02 (s)	17.1	0.75 (s)
27	25.9	1.27 (s)	25.9	1.13 (s)
28	179.9	-	182.9	-
29	32.9	0.94 (s)	32.6	0.90 (s)
30	23.6	1.06 (s)	23.6	0.93 (s)

Table 5. IC_{50} values from the antiplasmodial activity assays.

Samples	Pf3D7	PfDd2
	IC50 ± SD (µg/mL)	IC50 ± SD (µg/mL)
MDM	54.62 ± 0.12	ND
DMDM	11.98 ± 0.52	14.02 ± 0.17
F1DMDM	ND	ND
F2DMDM	37.51 ± 0.15	ND
F3DMDM	73.22 ± 1.06	ND
Stigmasterol	>50	ND
Spinasterol	>50	ND
Gluco-spinasterol	ND	ND
Oleanonic acid	>50	ND
Oleanolic acid	4.82 ± 0.42	7.02 ± 0.27
Positve controls	IC50 ± SD (nM)	IC50 ± SD (nM)
Artemisinin	16.61 ± 1.49	24.85 ± 1.49
Chloroquine	27.41 ± 0.16	284.50 ± 10.37

Note: MDM = Crude extract of methanol, DMDM = Crude extract of dichloromethane-methanol (1:1), F1DMDM = Hexane fraction, F2DMDM = Ethyl acetate fraction, F3DMDM = n-Butanol fraction, ND = Not Determined.

ppm (C-3) was absent, and a new signal appeared at δ_C = 77.8 ppm (C-3), characteristic of an oxymethine carbon. This confirms the reduction of the ketone to an alcohol.

Comparison of all these data with literature values led to the identification of compound (**5**) as oleanolic acid, isolated here for the first time from *D. microcarpum*. Oleanolic acid is a known hepatoprotective agent and exhibits antitumor and antiviral properties (34).

Antiparasitic Activity of *D. microcarpum* extracts

The evaluation of the antiplasmodial activity of the extracts and fractions obtained from *D. microcarpum* root bark (**Table 5**) revealed contrasting biological profiles depending on solvent polarity and the degree of fractionation.

Activity of Crude Extracts

The methanolic extract exhibited moderate activity (IC_{50} = 54.62 ± 0.12 µg/mL) against the chloroquino-sensitive strain (*Pf*3D7), indicating that the highly polar compounds present in this extract are not the main antiplasmodial metabolites. This observation is consistent with the fact that many active compounds on malaria parasites are semi-polar like flavonoids or triterpenes (36).

Crude extract of dichloromethane-methanol (1:1) (DMDM). This extract demonstrated strong activity against both strains, including the multi-resistant strain (*Pf*3D7: IC_{50} = 11.98 ± 0.52 µg/mL and *Pf*Dd2: IC_{50} = 14.02 ± 0.17 µg/mL). This suggests that the active metabolites are mainly compounds of intermediate polarity, probably the flavonoids or triterpenes detected through the phytochemical screening, which are known for their antiplasmodial activity.

Activity of Fraction

Ethyl Acetate Fraction (F_2DMDM). The ethyl acetate fraction showed strong activity (*Pf*3D7: IC_{50} = 37.51 ± 0.15 µg/mL), confirming that the semi-polar compounds present in the DMDM extract were partially concentrated in this fraction.

***n*-Butanol Fraction (F_3DMDM).** This fraction exhibited moderate activity (*Pf*3D7: IC_{50} = 73.22 ± 1.06 µg/mL), which is in line with its high content of tannins and saponins, compounds often associated with moderate to low antimalarial activity.

Activity of Compounds

Oleanolic acid demonstrated excellent activity against both strains (*Pf*3D7: IC_{50} = 4.82 ± 0.42 µg/mL and *Pf*Dd2: IC_{50} = 7.02 ± 0.27 µg/mL), displaying the best outcomes among all the samples tested. This supports the activity of the ethyl acetate fraction.

The results observed for artemisinin and chloroquine were in line with expectations, which validated the experimental conditions. Although natural extracts remain less active than pure molecules, the significant activity of oleanolic acid suggests real potential for new active compounds, especially due to its effectiveness against the resistant strain.

The activity observed against the multidrug-resistant *Pf*Dd2 strain enhances the value of *D. microcarpum* as a possible source of natural antimalarial compounds. The identified metabolite classes (flavonoids, triterpenes, sterols) correspond to those already known for their antiplasmodial properties.

Several studies have demonstrated that this triterpene possesses significant antimalarial activity, which is supported by the results obtained in the present study. Previous studies have shown that oleanolic acid exhibits IC_{50} values ranging from 3 to 15 µg/mL against *P. falciparum*, according to the strain (37, 38). Pentacyclic triterpenes, such as oleanolic acid and ursolic acid, disrupt the parasite's membrane and inhibit critical steps in its hemometabolism (39). Their efficacy against resistant strains is frequently reported, thus further highlighting their pharmacological value (40).

Several mechanisms of action of compounds on *P. falciparum* are being explored by scientists. The heme detoxification pathway is the most commonly investigated drug target in antimalarial research. 3-Hydroxy-11-keto-β-boswellic acid is (KBA) a triterpene isolated from *Boswellia serrata* that acts on *P. falciparum* by inhibiting heme detoxification pathways (41). KBA can induce the formation of reactive oxygen species (ROS), which leads to DNA fragmentation and protein carbonylation (41).

The results obtained in this research (IC_{50} < 5–7 µg/mL) are therefore among the highest recorded to date, demonstrating that the root of *D. microcarpum* is particularly rich in bioactive oleanolic acid.

Conclusion

This study aimed to evaluate the antiplasmodial activity of organic extracts, fractions, and isolated compounds from the root bark of *D. microcarpum* Guill. & Perr. , a species widely used in West African traditional medicine. The results clearly demonstrate the antimalarial potential of this plant, particularly its semi-polar extracts.

The high yields obtained with methanol and dichloromethane-methanol solvents reflect a rich content of extractable secondary metabolites. Phytochemical screening confirmed the presence of classes of bioactive compounds known for their antiparasitic properties, such as flavonoids, tannins, sterols, and triterpenes.

In vitro evaluation of antiplasmodial activity revealed that the dichloromethane-methanol extract (DMDM) and the ethyl acetate fraction (F_2DMDM) exhibited significant activity against *P. falciparum*. Of note, oleanolic acid demonstrated excellent activity *in vitro* against the *Pf*3D7 and *Pf*Dd2 strains, with IC_{50} values of 4.82 ± 0.42 µg/mL and 7.02 ± 0.27 µg/mL, respectively. This compound was isolated from the ethyl acetate fraction, which is the most active among the fractions.

The preserved activity against the multidrug-resistant *Pf*Dd2 strain is particularly significant in a context where the emergence of resistance to classical antimalarials constitutes a major public health challenge. *D. microcarpum*, and more specifically the isolated oleanolic acid, thus emerges as a promising source of novel natural antimalarial agents.

However, despite these encouraging results, further studies are required. It would be pertinent to continue the isolation of other metabolites present in the active fractions, to elucidate the mechanisms of action of oleanolic acid, to carry out cytotoxicity assays in order to evaluate their selectivity index. *In vivo* studies are needed to confirm the efficacy and safety profile of these

compounds as well as pharmacokinetic studies and investigations of pharmacoresistance.

Abbreviations

HSQC = Heteronuclear Single Quantum Coher ence; HMBC = Heteronuclear Multiple-Blond Coherence; IC_{50} = Inhibitory Concentration 50; NMR = Nuclear Magnetic Resonance; TLC = Thine Layer Chromatography; UV = Ultraviolet Visible; MDM: Crude extract of methanol; DMDM: Crude extract of dichloromethane-methanol (1: 1); F_1DMDM: Hexane fraction; F_2DMDM: Ethyl acetate fraction; F_3DMDM: *n*-Butanol fraction; CC: chronographic column; H/A: Hexane/Ethyl acetate; ND = Not Determined.

Declaration

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All regards to the authors of this study. It is the result of collaboration between the laboratories that participate in this work. During the preparation of this manuscript, the authors used ChatGPT-4 solely as an assistant for language editing, grammar correction, and improving readability. No data, figures, or original research concepts were generated using artificial intelligence. The authors thoroughly reviewed and edited all AI-assisted text, take full responsibility for the contents of the published article, and approved the final manuscript prior to submission.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics Statement

Not applicable.

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Supplementary Material

Supplementary Figure 1-**11** and **Supplementary Table 1**-**3** are provided in a single Microsoft Word document. The file can be downloaded from the following link.

References

1. Venkatesan P. The 2023 WHO World malaria report. The Lancet Microbe. 2024;5(3):e214. doi: https://doi.org/10.1016/s2666-5247(24)00016-8

2. DEP. Annuaire Statistique Sanitaire 2019-2020. Ministère de la Santé Publique et de la Population. 2020.

3. Uwimana A, Legrand E, Stokes BH, Ndikumana JLM, Warsame M, Umulisa N, *et al*. Emergence and clonal expansion of *in vitro* artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. Nat Med. 2020;26(10):1602-1608. doi: https://doi.org/10.1038/s41591-020-1005-2

4. Uwimana A, Umulisa N, Venkatesan M, Svigel SS, Zhou Z, Munyaneza T, *et al*. Association of *P. falciparum* kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. The Lancet Infectious Diseases. 2021;21(8):1120-1128. doi: https://doi.org/10.1016/s1473-3099(21)00142-0

5. Arzika II, Lobo NF, Lamine MM, Tidjani IA, Sandrine H, Sarrasin-Hubert V, *et al*. *P. falciparum* kelch13 polymorphisms identified after treatment failure with artemisinin-based combination therapy in Niger. Malar J. 2023;22(1):1-18. doi: https://doi.org/10.1186/s12936-023-04571-w

6. Yolidje I, Keita DA, Moussa I, Toumane A, Bakasso S, Saley K, *et al*. Enquête ethnobotanique sur les plantes utilisées traditionnellement au Niger dans la lutte contre les moustiques vecteurs des maladies parasitaires. Int. J. Bio. Chem. Sci. 2020;14(2):570-579. doi: https://doi.org/10.4314/ijbcs.v14i2.21

7. Kipre GR, Offoumou MR, Silue KD, Bouabre GM, Zirihi GN, Djaman AJ. Enquête ethnopharmacologique des plantes antipaludiques dans le département d'Agboville, sudest de la Cote d'Ivoire. J. App. Bioscience. 2017;109(1):10618. doi: https://doi.org/10.4314/jab.v109i1.6

8. Abdoulahi MII, Yanick Kevin MD, Lauve Rachel TY, Habibou HH, Sahabi B, Abdelkader AS, *et al*. Antibacterial Activity of Eight Medicinal Plants from the Traditional Pharmacopoeia of Niger. Journal of Tropical Medicine. 2023;2023(1):1-10. doi: https://doi.org/10.1155/2023/6120255

9. Idi IAM, Tali MBT, Tidjani IA, Habibou HH, Mbarga PE, Dize D, *et al*. Bio-guided investigation of nine Niger's ethnomedicinal plants reveals nature-derived antimalarial chemical pharmacophores for drug discovery. Chem Biodivers. 2025;22(11). doi: 10.1002/cbdv.202500879

10. Habibou HH, Abdoulahi MII, Khalid I. *In Vitro* Anti-*Shigella*, Antioxidant Activities, and Oral Acute Toxicity of Organics Extracts from the Root Bark of *Detarium microcarpum* Guill. and Perr. Journal of Tropical Medicine. 2024;2024(1):1-7. doi: https://doi.org/10.1155/2024/1330063

11. Daget P. Analyse d'ouvrage:*Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest*, de Michel Arbonnier. Acta Botanica Gallica. 2001;148(3):283-285. doi: https://doi.org/10.1080/12538078.2001.10515914

12. R. AB. The Useful Plants of West Tropical Africa. Nature. 1938;141(3558):54-54. doi: https://doi.org/10.1038/141054a0

13. Habibou HH, Abdoulahi MII, Moctar C, Zakari CO, Rahila HG, Khalid I. Phytochemistry and pharmacology activities of *D. microcarpum* (Fabaceae) used in the treatment of parasitic diseases in Niger: A review. J Pharmacogn Phytochem. 2022;11(2):60-67. doi: https://doi.org/10.22271/phyto.2022.v11.i2a.14377

14. Hama Hamadou H, Souley Kallo M, Maman Manzo L, Moussa I, Adamou R, Ikhiri K. Criblage phytochimique et dosage des polyphénols du *D. microcarpum* Guill. et Perr. utilisé dans le traitement des maladies parasitaires au Niger. Afrique SCIENCE. 2018;14(5).

15. Habibou HH, Fati AO, Abdoulahi MII, Abdelkarim SSS, Khalid I. A study on the anti-Shigella activity of extracts and constituents from the root bark of *D. microcarpum* (Fabaceae), a plant traditionally used to treat dysentery in Niger. Scientific African. 2025;30:e03067. doi: https://doi.org/10.1016/j.sciaf.2025.e03067

16. Nassirou R, Ibrahim M, Ilagouma A, Mahamadou A, Mamoudou M, Abdoulaye A, *et al*. Évaluation *in vitro* de l'activité antiplasmodiale d'extraits de plantes issues de la pharmacopée traditionnelle du Niger. J. App. Bioscience. 2015;89(1):8291. doi: https://doi.org/10.4314/jab.v89i1.8

17. S. FA, Burdet MM. Catalogue des périodiques de la bibliotheque des conservatoire et jardin botaniques de la ville de Genève. Taxon. 1981;30(2):546. doi: https://doi.org/10.2307/1220174

18. Hepper FN, Hutchinson J, Dalziel JM, Keay RWJ. Flora of West Tropical Africa. Kew Bull. 1954;9(3).

19. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman & Hall Pub. London, UK. 1998;

20. Evans WC. Trease and Evans pharmacognosy.(15thedn). WB Saunders and Company, London. 2002.

21. del Rayo CM, Phillipson JD, Croft SL, Yardley V, Solis PN. *In vitro* Antiprotozoal and Cytotoxic Activities of Some Alkaloids, Quinones, Flavonoids, and Coumarins. Planta med. 2004;70(1):70-72. doi: https://doi.org/10.1055/s-2004-815460

22. Trager W, Jensen JB. Human Malaria Parasites in Continuous Culture. Science. 1976;193(4254):673-675. doi: https://doi.org/10.1126/science.781840

23. Lambros C, Vanderberg JP. Synchronization of *P. falciparum* Erythrocytic Stages in Culture. The Journal of Parasitology. 1979;65(3):418. doi: https://doi.org/10.2307/3280287

24. Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. Simple and Inexpensive Fluorescence-Based Technique for High-Throughput Antimalarial Drug Screening. Antimicrob Agents Chemother. 2004;48(5). doi: 10.1128/AAC.48.5.1803-1806.2004

25. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. Journal of natural products. 2008;5.

26. Dabire CM, Bationo RK, Hema A, Nebie RCH, Pale E, Dhanabal SP, *et al*. Total phenolics content, flavonoids profiling and antioxidant activity of Lippia multiflora leaves extracts from Burkina Faso. Asian Journal of Plant Science and Research. 2015;5(5).

27. Zerbo P, Millogo Rasolodimby J, Nacoulma Ouedraogo O, Van Damme P. Plantes médicinales et pratiques médicales au Burkina Faso : cas des Sanan. Bois for. trop. 2011;307:41-53. doi: https://doi.org/10.19182/bft2011.307.a20481

28. Konaté K, Souza A, Thérèse KY, Dibala IC, Barro N, Millogo-Rasolodimby J, *et al*. Phytochemical composition, antioxidant and anti-inflammatory potential of bioactive fractions from extracts of three medicinal plants traditionally used to treat liver diseases in Burkina Faso. International Journal of Phytomedicine. 2011;3(3).

29. Habibou HH, Fati AO, Abdoulahi MII, Abdelkarim SSS, Khalid I. A study on the anti-Shigella activity of extracts and constituents from the root bark of *D. microcarpum* (Fabaceae), a plant traditionally used to treat dysentery in Niger. Sci Afr. 2025 Dec 1;30:1. doi: 10.1016/j.sciaf.2025.e03067

30. Cushnie TT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. International Journal of Antimicrobial Agents. 2011;38(2):99-107. doi: https://doi.org/10.1016/j.ijantimicag.2011.02.014

31. Ramalhete C, da Cruz FP, Lopes D, Mulhovo S, Rosário VE, Prudêncio M, *et al*. Triterpenoids as inhibitors of erythrocytic and liver stages of *Plasmodium* infections. Bioorganic & Medicinal Chemistry. 2011;19(24):7474-7481. doi: https://doi.org/10.1016/j.bmc.2011.10.044

32. Shu YZ. Recent Natural Products Based Drug Development: A Pharmaceutical Industry Perspective. J. Nat. Prod. 1998;61(8):1053-1071. doi: https://doi.org/10.1021/np9800102

33. Kayser O, Kiderlen AF, Croft SL. Natural products as

antiparasitic drugs. Vol. 90, Parasitology Research. 2003. doi: 10.1007/s00436-002-0768-3

34. Irungu BN, Orwa JA, Gruhnojic A, Fitzpatrick PA, Landberg G, Kimani F, *et al*. Constituents of the roots and leaves of Ekebergia capensis and their potential antiplasmodial and cytotoxic activities. Molecules. 2014;19(9). doi: 10.3390/molecules190914235

35. Abbas FA, Al-massarany SM, Khan S, Al-howiriny TA, Mossa JS, Abourashed EA. Phytochemical and biological studies on Saudi Commiphora opobalsamum L. Nat Prod Res. 2007;21(5). doi: 10.1080/14786410600942025

36. Moretti C, Sauvain M, Lavaud C, Massiot G, Bravo JA, Muñoz V. A Novel Antiprotozoal Aminosteroid from *Saracha punctata*. J. Nat. Prod. 1998;61(11):1390-1393. doi: https://doi.org/10.1021/np9800654

37. Kaur K, Jain M, Kaur T, Jain R. Antimalarials from nature. Vol. 17, Bioorganic and Medicinal Chemistry. 2009. doi: 10.1016/j.bmc.2009.02.050

38. Ayeleso TB, Matumba MG, Mukwevho E. Oleanolic acid and its derivatives: Biological activities and therapeutic potential in chronic diseases. Vol. 22, Molecules. 2017. doi: 10.3390/molecules22111915

39. Kuzminac IZ, Savić MP, Ajduković JovanaJ, Nikolić AR. Steroid and Triterpenoid Compounds with Antiparasitic Properties. Curr Top Med Chem. 2023;23(9). doi: 10.2174/1568026623666230126162419

40. Tajuddeen N, Van Heerden FR. Antiplasmodial natural products: An update. Vol. 18, Malaria Journal. 2019. doi: 10.1186/s12936-019-3026-1

41. Gupta M, Kumar S, Kumar R, Kumar A, Verma R, Darokar MP, *et al*. Inhibition of heme detoxification pathway in malaria parasite by 3-hydroxy-11-keto-β-boswellic acid isolated from *Boswellia serrata*. Biomedicine and Pharmacotherapy. 2021 Dec 1;144. doi: 10.1016/j.biopha.2021.112302

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