In vitro antiplasmodial activity-directed investigation and UPLC–MS fingerprint of promising extracts and fractions from *Terminalia ivorensis* A. Chev. and *Terminalia brownii* Fresen

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Abstract

Ethnopharmacological significance: Medicinal plants from the *Terminalia* genus are widely used as remedies against many infectious diseases, including malaria. As such, *Terminalia ivorensis* A. Chev. and *Terminalia brownii* Fresen. are famous due to their usefulness in traditional medicines to treat malaria and yellow fever. However, further information is needed on the extent of anti-*Plasmodium* potency of extracts and fractions from these plants and their phytochemical profile.

Aim of the study: This study was designed to investigate the *in vitro* antiplasmodial activity and to determine the chemical profile of promising extracts and fractions from *T. ivorensis* and *T. brownii* stem bark.

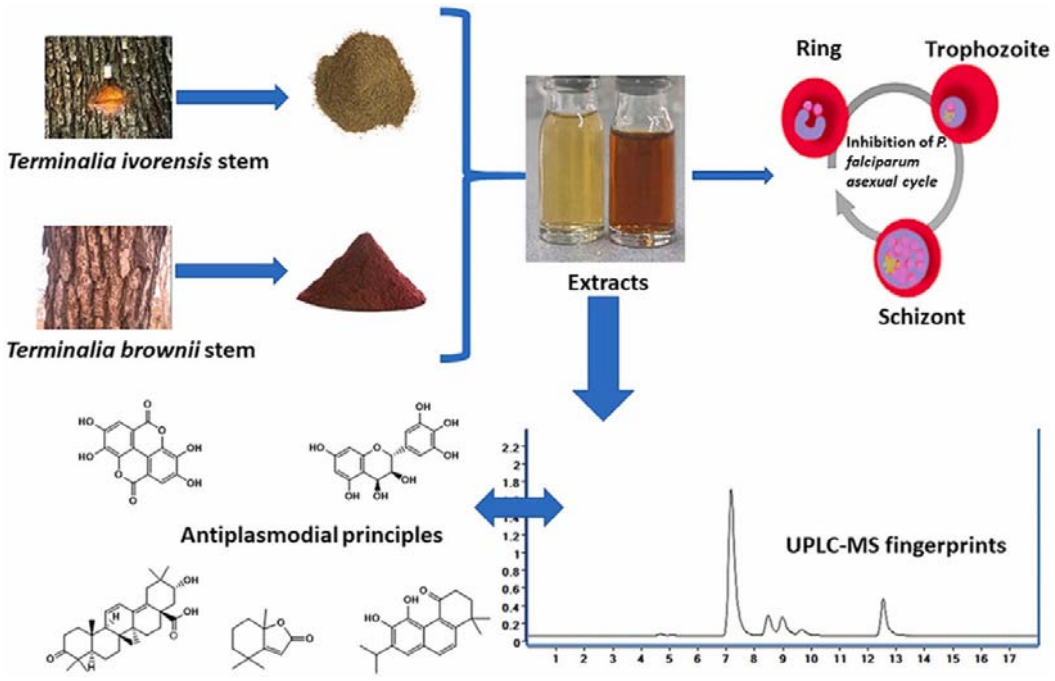
Materials and methods: Crude aqueous, ethanolic, methanolic, hydroethanolic and ethyl acetate extracts were prepared by maceration from the stem barks of *T. brownii* and *T. ivorensis*. They were subsequently tested against chloroquine-sensitive (*Pf*3D7) and multidrug-resistant (*Pf*Dd2) strains of *P. falciparum* using the parasite lactate dehydrogenase (*Pf*LDH) assay. Extracts showing very good activity on both plasmodial strains were further fractionated using column chromatography guided by evidence of antiplasmodial activity. All bioactive extracts and fractions were screened for their cytotoxicity on Vero and Raw cell

lines using the *resazurin*-based assay and on erythrocytes using the hemolysis assay. The phytochemical profiles of selected potent extracts and fractions were determined by UPLC–QTOF-MS analysis.

Results: Of the ten extracts obtained from both plant species, nine showed inhibitory activity against both *P. falciparum* strains (*Pf*3D7 and *Pf*Dd2), with median inhibitory concentration (IC_{50}) values ranging from 0.13 µg/ml to 10.59 µg/ml. Interestingly, the aqueous extract of *T. ivorensis* (Ti^{W}) and methanolic extract of *T. brownii* (Tb^{M}) displayed higher antiplasmodial activities against both strains (IC_{50} 0.13–1.43 µg/ml) and high selectivity indices (SI > 100). Their fractionation led to two fractions from *T. ivorensis* and two from *T. brownii* that showed very promising antiplasmodial activity (IC_{50} 0.15–1.73 µg/mL) and SI greater than 100. The hemolytic assay confirmed the safety of crude extracts and fractions on erythrocytes. UPLC–MS-based phytochemical analysis of the crude aqueous extract of *T. ivorensis* showed the presence of ellagic acid (**1**) and leucodelphidin (**2**), while analysis of the crude methanol extract of *T. brownii* showed the presence of ellagic acid (**1**), leucodelphinidin (**2**), papyriogenin D (**3**), dihydroactinidiolide (**4**) and miltiodiol (**5**).

Conclusions: The extracts and fractions from *T. ivorensis* and *T. brownii* showed very good antiplasmodial activity, thus supporting the traditional use of the two plants in the treatment of malaria. Chemical profiling of the extracts and fractions led to the identification of chemical markers and the known antimalarial compound ellagic acid. Further isolation and testing of other pure compounds from the active fractions could lead to the identification of potent antiplasmodial compounds.

Graphical abstract



Keywords: *Plasmodium falciparum;* Antiplasmodial; *Terminalia ivorensis; Terminalia brownie;* UPLC–MS fingerprints; Fractionation

1. List of abbreviations

CC_{50}	Median cell cytotoxic concentration
IC_{50}	Median inhibitory concentration
Art	Artemisinin
CQ	Chloroquine
Chl	Methylene chloride
MeOH	Methanol
Hex	Hexane
Ea	Ethyl acetate
Ti	*Terminalia ivorensis*
Tb	*Terminalia brownii*
W	Water
R3	Residue
RC	Raw Cells
VC	Vero cells
SD	Standard Deviation
SI	Selectivity Index
RI	Resistance Index
E	Ethanol
H-E	Hydro-Ethanol
Pf	*Plasmodium falciparum*
IC_{50}*Pf*Dd2	Median Inhibitory Concentration against *Plasmodium falciparum* Dd2 strain
IC_{50}*Pf*3D7	Median Inhibitory Concentration against *Plasmodium falciparum* 3D7 strain
QTOF	Quqdrupole Time of Fly
UPLC	Ultraperformance liquid chromatography
MS	Mass Spectrometry
RPMI1640	Roswell Park Memorial Institute medium
***Pf*LDH**	*Plasmodium falciparum* Lactate Dehydrogenase
ACTs	Artemisinin-Based Combination Therapies
SI_{Pf3D7}	Selectivity index toward *Plasmodium falciparum* 3D7 strain
SI_{PfDd2}	Selectivity index toward *Plasmodium falciparum* Dd2 strain

APAD	acetylpyridine adenine dinucleotide
NBT	Nitrotetrazolium blue chloride
PES	Phenazine ethosulphate
DMSO	dimethyl sulfoxide

1. Introduction

Despite great efforts to control and eliminate malaria, it is still one of the major causes of death and poverty in Africa. It disproportionately affects vulnerable groups, including women and children, particularly from the poorest households (WHO, 2021a, WHO, 2021b). Moreover, endemic countries are facing the double challenge of protecting their citizens from existing threats to public health, such as malaria, and emerging ones, such as COVID-19. In 2020, nearly half of the world's population was at risk of malaria. Most cases and deaths occur in sub-Saharan Africa. However, the WHO regions of South-East Asia, Eastern Mediterranean, Western Pacific, and the Americas also report significant numbers of cases and deaths (WHO, 2021a, WHO, 2021b). There were an estimated 241 million cases of malaria in 2020, and the estimated number of malaria deaths stood at 627 000 (WHO, 2021a, WHO, 2021b). The WHO African Region carries a disproportionately high share of the global malaria burden. In 2020, the region was home to 95% and 96% of malaria cases and deaths, respectively. Children under 5 years of age are the most vulnerable group affected by malaria, as in 2020, they accounted for approximately 80% of all malaria deaths in the WHO African Region (WHO, 2020).

The WHO Global Technical Strategy for Malaria 2016–2030 (GTS) includes a technical framework for all malaria-endemic countries with the “High Burden to High Impact” approach to be supported by WHO, the RBM Partnership to End Malaria and other partners, and implemented at the country level. Goals for 2020 included reaching at least a 40% reduction in malaria incidence and death rates and the elimination of malaria in at least 10 countries (WHO, 2015). However, these milestones were not met, and the advent of the COVID-19 pandemic further complicated the situation with a significantly negative impact on malaria control, and other major killers as major resources were diverted to the new threat. Additionally, the fear of being contaminated or diagnosed positive for coronavirus has led malaria patients to be reluctant to attend health centers.

More recently, the World Health Organization (WHO, 2021a, WHO, 2021b) recommended the widespread immunization of children across sub-Saharan Africa and elsewhere with the world's first RTS, S vaccine against malaria (WHO, 2021a, WHO, 2021b). However, it is important to note that this vaccine does not offer full protection against malaria, as it reduces the risk of contracting malaria by only 40% and the risk of hospital admission with severe malaria by approximately 30%. Hence, the WHO is right in saying “At the same time, we acknowledge that this is not a silver bullet solution, and we must continue to develop new and more effective therapies, vaccines and preventive tools against malaria (MMV, 2021).

Existing malaria treatment in Africa targets potentially drug-resistant organisms using artemisinin-based combination therapies (ACTs). One of the most serious threats to malaria control has been resistance to ACTs in the Greater Mekong Subregion. If artemisinin-resistant strains of *P. falciparum* arise in or are imported to Africa, it would be catastrophic for malaria control on the African continent. Of note, a high frequency of unexplained slow parasite clearance times has been reported among Ugandan children treated with intravenous artesunate for severe malaria (Asua et al., 2021; Rosenthal, 2021). Throughout East Africa, residual submicroscopic parasitemia after ACT treatment has also been reported (Kibwika et al., 2018). Further driving drug resistance is the wide availability of substandard and counterfeit malaria treatments in affected countries. Emphasis must therefore be placed on the search for alternative active agents that can replace ACTs in the event of widespread resistance.

Of particular concern, the major hindrance to malaria control remains the paucity of new drug candidates endowing novel mechanisms of action. In that framework, natural products have proven to be valuable sources of antiplasmodial agents since the discovery of the first antimalarial drug in the 1800s (Newman and Cragg, 2016**).** In this direction, widely distributed *Terminalia* spp. such as *Terminalia ivorensis* A. Chev. and *Terminalia brownii* Fresen. (Combretaceae) are famous due to their usefulness in traditional medicines to treat malaria and yellow fever **(**Liu et al., 2009; Mosango, 2013). Of note, *Terminalia ivorensis* (local name: black afara- Cameroon) is a large deciduous forest tree ranging in height from 15 to 46 m, branchless for up to 30 m. Bole clean, very straight with small buttresses and sometimes fluted and distributed in West and Central Africa (Foli, 2009). In addition, *Terminalia brownii* (local names: kuuku, muvuku, koloswa, ibukoi- Kenya), weba- Ethiopia, orbukoi, maasai- Tanzania), and mbarao or mwalambe- Kiswahili) is a leafy deciduous tree with an attractive somewhat layered appearance, usually 4–15 m or up to 25 m high with a rounded, flat-topped, spreading crown and a straight bole; branches reaching close to the ground. Slash dull red–brown, the bark of branchlets gray fibrous. Young bark smooth, whitish, old bark gray, longitudinally fissured, young shoots densely hairy (Foli, 2009). Previous phytochemical investigations were conducted on both plant species, resulting in the identification of terpenes, tannins, flavonoids, lignans and simple phenols (Zhang et al., 2019; Salih et al., 2017; Yamauchi et al., 2016; Negishi et al., 2011; Ponou et al., 2011, 2010). In addition, crude extracts from *T. brownii* were reported to have activity against malaria parasites (Biruk et al., 2020; Machumi et al., 2013**)**.

However, previous works conducted on the two plants mainly described their phytochemical profiles and, in some cases, random testing of extracts/compounds for biological activity. No previous study has been conducted on these plants using a rational approach in portraying the bioactivities based on potency-directed choice of extracts or fractions to be further investigated. We anticipated that the achievements from a bioguided strategy would set a strong basis for future investigation of the promising fractions toward the isolation of pure active principles that might be flushed into the medicinal chemistry pipeline. Hence, this study provides an elucidation of the antiplasmodial potency of fractions afforded from potent *T. ivorensis* and *T. brownii* extracts using a bioguided approach and their phytochemical profile using UPLC–MS fingerprints.

2. Materials and Methods

2.1. General experimental procedures

A Waters UPLC and Waters Synapt G2 QTOF Mass Spectrometer System including MassLynx v4.1 software was used for analyzing the samples. For UPLC method development, a 1.7 μm ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm) was used. The gradient mobile phases used were A, water +0.1% HCO2H; and B, methanol +0.1% HCO2H. The elution flow rate was 0.300 mL/min. Elution started with 97% A and 3% B; a linear gradient to 100% B until 14 min; isocratic from 14 to 16 min with 0% A and 100% B; and a linear gradient from 16 to 20 min to reach 97% A and 3% B at completion.

2.2. Reagents, chemicals and materials suppliers

Chloroquine phosphate, artemisinin, glucose, HEPES, Giemsa, hypoxanthine, RPMI-1640, sodium bicarbonate, sorbitol, Giemsa stain, DMSO and flat-bottomed 96-well plates were sourced from Sigma Chemicals (Sigma–Aldrich, Germany). Phosphate buffered saline (PBS),

fetal bovine serum (FBS), Albumax II, Dulbecco's Modified Eagle Medium (DMEM), EDTA, Saponin, Triton X-100, and gentamicin were purchased from Gibco (Gibco, Waltham, MA, USA). APAD and NBT/PES were purchased from Merck (Merck, Germany). Silica gel for column chromatography (60–230 mesh) was supplied by Merck (Merck, Darmstadt, Germany). Dichloromethane and methanol were procured from Brenntag (Brenntag, Essen, Germany), while ethyl acetate was obtained from Solventis (Solventis, Guildford, United Kingdom).

2.3. Plant collection and extraction

The stem barks of *T. ivorensis* and *T. brownii* were harvested in August 2017 at Carrefour MEEC-Nkolbisson, Yaounde-Cameroon, 3°52′00.0” N 11°3100.1″ E. The Plant List (http://www.theplantlist.org) was used to check the accuracy of plant names, and botanical identification was confirmed at the National Herbarium of Cameroon, Yaoundé, where voucher specimens are deposited under the reference numbers 48878/HNC and 36394/HNC for *T. ivorensis* and *T. brownii,* respectively. The collected samples were dried under shade at room temperature (25–29 °C) for 2 weeks and ground to fine powders (Ø∼0.5 μm) using a miller (Hammer Mill, Leabon 9FQ, Zhengzhou, PRC) before extraction. Each powder (1.5 kg) was separately macerated in 4.5 L of ethanol, methanol, ethyl acetate, hydroethanol (70%) and distilled water for three consecutive days. The macerates were then filtered using Whatman No 1 filter paper, and the residues were macerated afresh for two consecutive days and treated similarly. The filtrates were pooled and evaporated using a rotary evaporator (Rotavapor, BUCHI 071, Switzerland) at 65 °C and 75 °C for alcoholic and ethyl acetate extracts, respectively. The aqueous extracts were lyophilized using a Virtis Wizard 2.0 Freeze Dryer Lyophilizer: Model: XLS-70. The extraction yields were determined with regard to the initial weight of individual powders, and the dried crude extracts (greenish-brown color) were subsequently tested for antiplasmodial activity.

2.4. Fractionation of biologically active extracts

This study identified promising extracts [IC_{50} < 1 μg/mL; selectivity indices (Vero and Raw 264.7-cell lines) > 10-fold; and harmless toward erythrocytes) that were subsequently submitted to bioactivity-guided fractionation. Following these criteria, the aqueous and methanolic extracts from the stem barks of *T. ivorensis* (a) and *T. brownii* (b), which showed the best antiplasmodial activity and good selectivity, respectively, were fractionated using liquid–liquid partitioning (a) according to the procedure described by Xie et al. (2011) and flash chromatography (b). Briefly, 90 g of *T. ivorensis* aqueous extract was suspended in 5% MeOH/H_2O and then successively extracted with n-hexane, dichloromethane and ethyl acetate (Fig. 1A). Each fraction was evaporated under reduced pressure at 45–55 °C and then lyophilized. Three main residues were obtained and named fractions dichloromethane fraction (Ti^WChl) [23.0 g, yield 23.0%], ethyl acetate fraction (Ti^WEa) [15.7 g, yield 15.7%] and the remaining aqueous residue fraction (Ti^WR3) [48.5 g, yield 48.5%]. Partitioning using hexane resulted in a nonquantifiable residue that was not further investigated. The methanolic extract of the stem bark of *T. brownii* did not dissolve in 5% MeOH/H_2O and was therefore fractionated using flash chromatography (Fig. 1B). A portion of 75 g of the extract was dissolved in methanol and adsorbed onto an equivalent mass of silica gel. The dry mixture of crude extract-silica gel was further subjected to flash column chromatography (CC) using silica gel (70–230 mesh, 60 Å, Aldrich) as the stationary phase and gradient elution with hexane/ethyl acetate and ethyl acetate/methanol at different concentrations to yield 76 fractions (ca. 250 mL each), which were combined into 5 major fractions [Tb01 –Tb05]

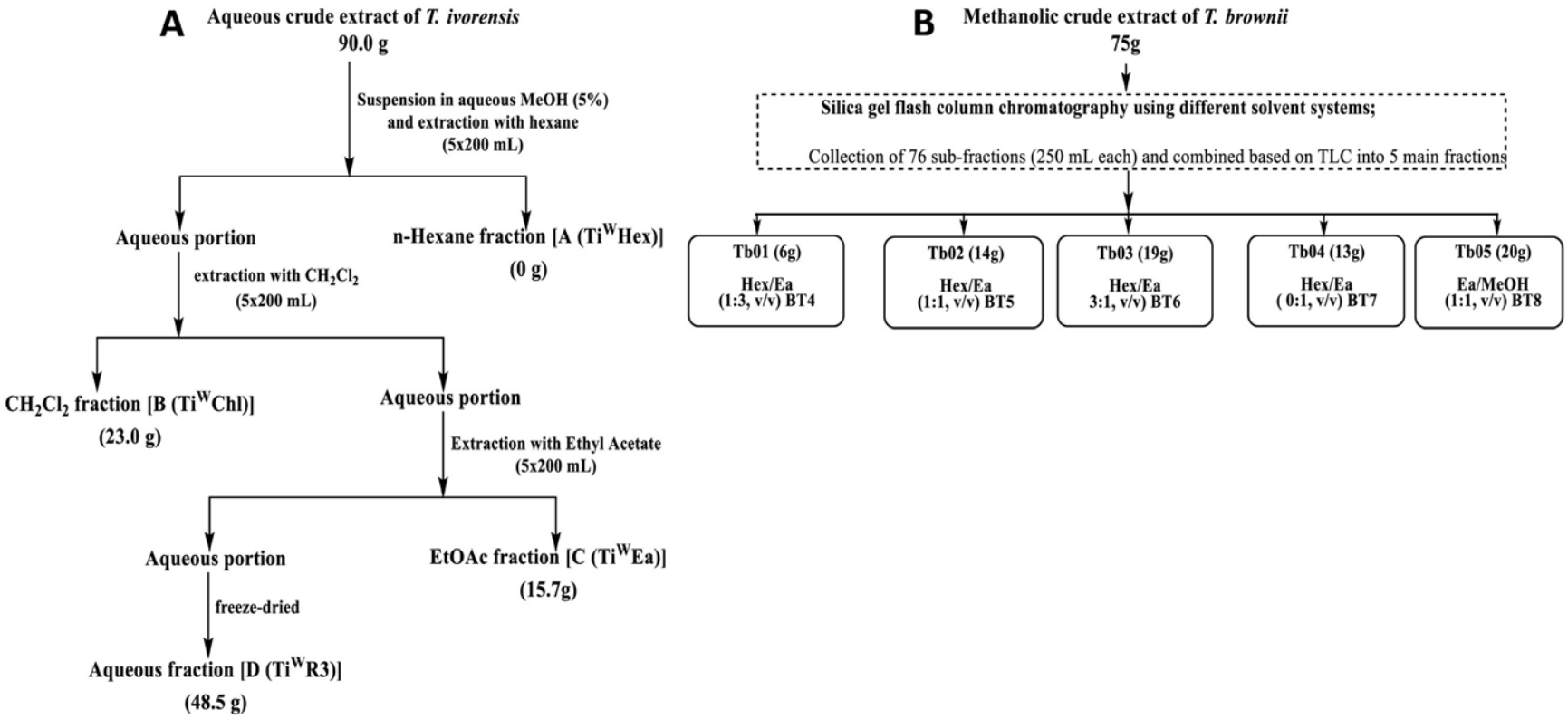


Fig. 1. Fractionation procedures of *T. ivorensis* aqueous crude extract (A) and *T. brownii* methanolic crude extract (B). A: 5% MeOH/H_2O (v/v) solution of *T. ivorensis* aqueous extract was successfully exhausted with n-hexane, dichloromethane and ethyl acetate; B: *T. brownii* methanol extract was submitted to flash column chromatography using silica gel and gradient elution with hexane/ethyl acetate and ethyl acetate/methanol at different concentrations.

based on their thin layer chromatography (TLC) profiles. Each of the afforded fractions from both plant extracts was tested for antiplasmodial activity.

2.5. Sample preparation for biological assays

A stock solution of each extract and fraction was prepared at 100 mg/mL in 100% DMSO (Sigma–Aldrich, Munich, Germany). Master plates were then prepared by mixing 2 µL of each stock solution with 198 µL of fresh incomplete RPMI 1640 culture medium to yield a concentration of 1 mg/mL (1% DMSO) followed by a 5-fold serial dilution. Chloroquine (CQ) and artemisinin (Art) (Sigma–Aldrich, Munich, Germany) used as standard drugs were prepared at 1 mM in sterile distilled water and 100% DMSO, respectively. In assay plates, the final concentration of DMSO was ≤0.5%. Extracts and fractions were tested at concentrations ranging from 0.16 to 100 µg/mL, and chloroquine and artemisinin were included at the highest concentration of 1 µM.

2.6. Determination of the hemolytic potential of extracts and fractions

Hemolysis assays were performed as described by Kazi et al. (1994) against normal O^+ human red blood cells (RBCs) to assess the hemolytic effect of the tested extracts and fractions prior to the antiplasmodial assay at concentrations ranging from 250 to 15.625 µg/mL. Five hundred microliters of each tested extract and fraction were added to 500 µL of RBCs at 2% hematocrit, and the mixture was incubated at 37 °C for 2 h. Controls were Triton X-100 0.1% (v/v) (positive) and phosphate-buffered saline (PBS-negative control) (Sigma–Aldrich, Munich, Germany), corresponding to 100% and 0% hemolysis, respectively. After incubation, the assay mixture was centrifuged at 1500 rpm for 5 min, and the amount of hemoglobin released was quantified spectrophotometrically in the supernatant at 540 nm using a Magelan Infinite M200 plate reader (Tecan) and compared to the negative control.

2.7. Determination of the in vitro antiplasmodial activity of plant extracts and fractions

2.7.1. Parasite maintenance

P. falciparum 3D7 and Dd2 strains obtained from BEI Resources (https://www.beiresources.org/) were maintained in culture as described by Trager and Jensen (1976) in fresh O-positive human red blood cells suspended at 4% (V/V) hematocrit in complete RPMI 1640 medium (500 mL RPMI 1640 (Sigma, Munich, Germany) supplemented with 25 mM HEPES, 10% Albumax II (Gibco, Waltham, MA, USA), 1X hypoxanthine (Gibco, Waltham, MA, USA) and 50 mg/mL gentamicin (Sigma–Aldrich, Munich, Germany) and incubated at 37 °C in a humidified atmosphere of 5% CO_2. The medium was renewed daily to propagate the culture. Parasite growth was monitored by microscopic examination of Giemsa-stained thin blood smears under oil immersion.

2.7.2. Synchronization of P. falciparum asexual-blood stage culture

To obtain the ring stage of the parasite, the cultures were synchronized as previously described **(**Lambros & Vanderberg, 1979**)**. Briefly, the cultures with parasites mainly at the ring stage were treated with an equal volume of aqueous 5% D-sorbitol for 10 min. The treated cultures were centrifuged at 1500 rpm for 5 min, and the pellets were further

suspended in complete medium. The synchronized culture was used at 2% parasitemia and 1% hematocrit for the antiplasmodial assay.

*2.7.3. Pf*LDH-based *antiplasmodial* assay

Crude extracts and fractions were evaluated for their inhibitory potential against *P. falciparum* using the *Pf*LDH assay as described by Makler and Hinrichs (1993). Essentially, in 96-well flat-bottomed microtiter plates, 10 µL of each inhibitor from the master plates was added to triplicate wells, followed by 90 µL of synchronized parasites (ring stage) at 2% parasitemia and 1% hematocrit. The final test concentrations of extracts ranged from 0.16 to 100 µg/mL, and the final concentration of DMSO in wells did not exceed 0.5%. The negative control consisted of 0.5% DMSO, while the standard drugs (CQ and Art) were tested as positive controls with the highest concentration of 1 µM. Plates were then incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Thereafter, 20 µL of culture from each well was transferred to a new plate containing 100 µL of Malstat solution **[**55 mM Tris, 0.22 M L-lactic acid, 0.17 mM acetylpyridine adenine dinucleotide [APAD; Sigma–Aldrich, Germany], 0.2% [v/v] Triton X-100 (Sigma–Aldrich, Germany), pH 9.0**],** and 25 µl NBT/PES solution (1.96 mM nitrotetrazolium blue chloride, 0.24 mM phenazine ethosulphate) (Sigma–Aldrich, Germany). The plate was left to develop in the dark for 30 min at room temperature before recording the absorbance at 650 nm using a Magellan Infinite M200 plate reader (Tecan, Germany). These data were normalized to percent control activity using Microsoft Excel software, and median inhibitory concentrations (IC_{50}s) were calculated using Prism 5.0 software (GraphPad) with data fitted by nonlinear regression to the variable slope sigmoidal dose–response formula $y = 100/[1 + 10^{(\log IC50 - x)H}]$, where H is the Hill coefficient or slope factor (Singh and Rosenthal, 2001).

The data analysis was performed with GraphPad Prism 5.0 software, fitting by nonlinear regression, and concentration–response curves were generated to determine the median inhibitory concentration (IC_{50}) for each inhibitor.

The resistance index (RI) was determined as the ratio of the IC_{50}-resistant strain to the IC_{50}-sensitive strain**.** RI values below 1 indicated inhibitors preferentially acting against the resistant strain and *vice versa*.

2.8. Determination of the cytotoxicity of *Plasmodium* inhibitors

Vero ATCC CRL 1586 (African green monkey kidney) and Raw 264.7 (macrophage) cells were grown in a T-25 cm^2 flask containing DMEM (Sigma–Aldrich, Munich, Germany) supplemented with 2 mM L-glutamine (Sigma–Aldrich, Munich, Germany) and 10% fetal bovine serum (ATCC) and maintained at 37 °C in a humidified incubator containing 5% CO_2. For the experiments, 80–90% confluent cell culture was trypsinized and counted using a Neubeaur cell counter and seeded onto 96-well clear flat-bottomed plates treated for cell culture (Costar, USA) at a cell density of 10^4 cells/well and allowed to adhere overnight. Cells were then treated with serially diluted concentrations of antiplasmodial crude extracts and fractions for 48 h. Afterwards, 10 µL of resazurin solution (0. 15 mg/mL prepared in PBS) was added to each well, and the plates were reincubated for 4 h. Of note, resazurin is a nontoxic, cell-permeable compound that utilizes the reducing power of living cells to detect cell viability by converting from a nonfluorescent blue dye to the highly fluorescent red dye resorufin in response to changes in the reducing environment within the cytosol of the cell. The level of fluorescence that positively correlates with cell viability was measured using a

Magellan Infinite M200 plate reader (Tecan, Germany) at an excitation wavelength of 530 nm and an emission wavelength of 590 nm. The inhibitory percentage of cell proliferation was calculated with regard to the negative control. The concentration–response curves were plotted using inhibitory percentages versus the logarithm of the drug concentration to determine the concentration of drug that reduces cell viability by 50% (CC_{50}) using GraphPad Prism 5.0 software. Selectivity indices (SI) [$SI = CC_{50}/IC_{50}$] were determined for each inhibitor based on their antiplasmodial activity (IC_{50}) and cell cytotoxicity (CC_{50} on Vero and Raw cells).

2.9. Qualitative chemical profiling of extracts and fractions using Ultra performance liquid chromatography quadrupole time of fly mass spectrometry (UPLC-QTOF-MS)

2.9.1. Sample preparation

Extracts and fractions were separately dissolved in HPLC grade methanol and purified water at a ratio of 1:1. The samples were subjected to sonication and centrifugation before transfer to HPLC vials. Each sample (1 mg/ml) was prepared for UPLC–MS analysis.

2.9.2. UPLC-QTOF-MS analysis

A Waters UPLC and Waters Synapt G2 QTOF Mass Spectrometer System including MassLynx v4.1 software was used for analyzing the samples following the general experimental procedures described above. For mass detection, both positive and negative ionization modes were used.

MS ESI + parameters: source (capillary (kv) 2.6000, sampling cone [20.0000], extraction cone [4.0000]; temperature (source [120], desolvation [350]); gas flow (cone gas (L/h) [10.0], desolvation [650]; mass range (low mass [50], high mass [1200]).

MS ESI- parameters: source (capillary (kv) 2.0000, sampling cone [25.0000], extraction cone [4.0000]; temperature (source [120], desolvation [300]); gas flow (cone gas (L/h) [10.0], desolvation [600]; mass range (low mass [50], high mass [1200]).

2.9.3. Identification of compounds

Compounds were tentatively identified by generating molecular formulas from MassLynx V 4.1 based on their iFit value and by comparison of the MS/MS fragmentation pattern with that of matching compounds from Waters UNIFI® Scientific Information System (version 1.9.2) accessing the Chinese Natural Products database. Ellagic acid (14668-50 MG) analytical standards were purchased from Sigma–Aldrich (Pty) LTD, South Africa, to confirm the activity and presence of the compound in *T. ivorensis* and *T. brownii* plant extracts.

3. Results

3.1. Prior evaluation of the hemolytic potential of extracts and fractions

The potential of crude extracts and fractions from *T. ivorensis* and *T. brownii* to elicit hemolysis was assessed on fresh erythrocytes. The results revealed no hemolytic effect at up

to 250 µg/mL extracts and fractions, suggesting that the activity detected at concentrations below this threshold was due to the effect of inhibitors alone.

3.2. Biological activities of extracts and fractions

3.2.1. In vitro activities of crude extracts from T. ivorensis and T. brownii

A total of 10 plant extracts from *T. brownii* and *T. ivorensis* were tested against chloroquine-sensitive (*Pf*3D7) and multidrug-resistant (*Pf*Dd2) strains of *P. falciparum*. Overall, all the crude extracts inhibited the growth of both parasite strains with median inhibitory concentrations (IC_{50}) ranging from 0.13 µg/mL to 10.59 µg/mL, except for the hydroethanol extract of *T. ivorensis*, which preferentially inhibited *Pf*Dd2 (IC_{50} = 0.85 µg/mL) but not *Pf*3D7 up to 100 µg/mL (Table 1, Fig. 2). Based on the antiplasmodial activity classification criteria for plant products by Muganza et al. (2016), 8 extracts out of the 10 tested, namely, Ti^{E}, Ti^{H-E}, $Ti^{M,}$ and Ti^{EA} from *T. ivorensis* and Tb^{E}, Tb^{M}, $Tb^{W,\ and}$ Tb^{H-E} from *T. brownii,* showed highly potent activities against both *P. falciparum* strains with IC_{50} values below 3 µg/mL (Table 1). Importantly, two extracts (Ti^{W} and Tb^{M}) exhibited higher inhibitory effects on both *Pf*3D7 and *Pf*Dd2 with IC_{50} < 1 µg/mL, coupled with higher selectivity indices (SI) ranging from >255 to > 1925 relative to Vero and Raw 264.7-cell lines. These activity parameters make the two extracts more attractive for further investigation.

The resistance indices (RI = IC_{50}*Pf*Dd2/IC_{50}*Pf*3D7) for the frontrunner extracts (Ti^{W} and Tb^{M}) were 7.53 and 5.46 (Table 1), indicating fundamental differences in the biology of both *P. falciparum* strains used in this study.

Table 1. Antiplasmodial activity and selectivity of *T. ivorensis* and *T. brownii* crude extracts.

Species	Code	Antiplasmodial activity		RI	**Cell Cytotoxicity		***Selectivity index			
		*IC_{50} ± SD (µg/mL)			VC	RC	(SI_{VC})		(SI_{RC})	
		*Pf*3D7	*Pf* Dd2		CC_{50} ± SD (µg/mL)		*Pf*3D7	*Pf*Dd2	*Pf*3D7	*Pf*Dd2
T. ivorensis	Ti^{W}	0.13 ± 0.02	0.98 ± 0.06	7.53	>250	>250	>1923	>255	>1923	>255
	Ti^{E}	0.83 ± 0.01	1.44 ± 0.11	1.73	>250	>250	>301	>173	>301	>173
	Ti^{H-E}	>100	0.85 ± 0.10	–	>250	>250	>2.5	>294	>2.5	>294
	Ti^{M}	0.86 ± 0.01	1.02 ± 0.05	1.18	>250	>250	>291	>245	>291	>245
	Ti^{Ea}	1.74 ± 0.06	1.04 ± 0.07	0.5	198.3 ± 0.10	108.8 ± 0.05	114	191	62	105
T. brownii	Tb^{W}	1.07 ± 0.01	1.07 ± 0.09	1	>250	>250	>233	>233	>233	>233
	Tb^{E}	0.96 ± 0.02	2.23 ± 0.12	2.32	>250	>250	>260	>112	>260	>112
	Tb^{H-E}	0.91 ± 0.02	0.93 ± 0.03	1.02	>250	>250	>274	>268	>274	>268
	Tb^{M}	0.13 ± 0.00	0.71 ± 0.02	5.46	>250	>250	>1923	>352	>1923	>352
	Tb^{Ea}	10.59 ± 0.30	6.28 ± 0.95	0.59	>250	116.1 ± 2.97	>23	>39	11	18
Reference Drugs	Artemisinin	3.38 ± 0.066 (nM)	14. 47 ± 0.02 (nM)	4.3						
	Chloroquine	7.55 ± 0.01 (nM)	133 ± 0.02 (nM)	17.61						

Inhibitors were tested against parasites in culture; *IC_{50}: Median inhibitory concentration; Cytotoxicity of compounds was tested against normal mammalian cells, **CC_{50}: Median cell cytotoxic concentration; ***Selectivity values represent the ratio of CC_{50} to IC_{50}; RI: IC_{50}*Pf*Dd2/IC_{50}*Pf*3D7; Data are mean values from triplicate experiments; *Ti: Terminalia ivorensis; Tb*: *Terminalia brownii;* W*:* Water; E: Ethanol; H-E: Hydro-Ethanol; M: Methanol; Ea: Ethyl acetate; Art: Artemisinin; CQ: Chloroquine; RC: Raw Cells:, VC: Vero cells:, SD: Standard Deviation, SI: Selectivity Index, RI: Resistance Index.

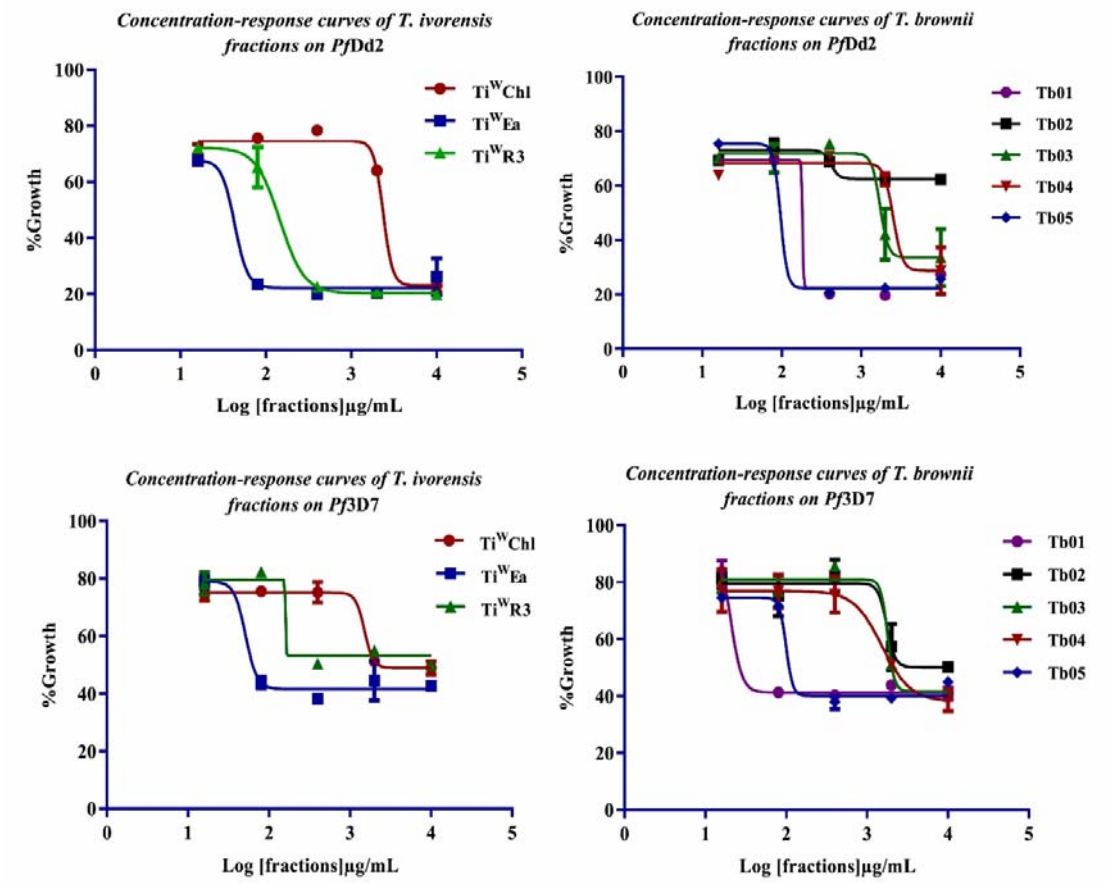


Fig. 2. Concentration–response curves of *T. ivorensis* and *T. brownii* crude extracts on *P. falciparum* Dd2 and 3D7. Data were normalized to percent control activity and median inhibitory concentrations (IC_{50}s) calculated using Prism 5.0 software (GraphPad) with data fitted by nonlinear regression to the variable slope sigmoidal dose–response formula $y = 100/[\ 1 + 10^{(\log IC50 - x)H}]$, where *H* is the Hill coefficient or slope factor. Ti^{W}: aqueous extract of *T. ivorensis*; Ti^{E}: ethanol extract of *T. ivorensis*; Ti^{H-E}: hydroethanol (70%) extract of *T. ivorensis*; Ti^{M}: methanol extract of *T. ivorensis*; Ti^{Ea}: ethyl acetate extract of *T. ivorensis*; Tb^{W}: aqueous extract of *T. brownii*; Tb^{E}: ethanol extract of *T. brownii*; Tb^{H-E}: hydroethanol (70%) extract of *T. brownii*; Tb^{M}: methanol extract of *T. brownii*; Tb^{Ea}: ethyl acetate extract of *T. brownii.*

3.2.2. In vitro antiplasmodial activity of fractions from T. ivorensis and T. brownii

Two extracts (Ti^{W} and Tb^{M}) displaying the highest potency and selectivity against both multidrug-resistant (Dd2) and chloroquine-sensitive (3D7) strains of *P. falciparum* (IC_{50} < 1 µg/mL; SI > 250) were subjected to liquid–liquid partitioning for the aqueous crude extract of *T. ivorensis* to yield three main fractions (Ti^{W}Chl; Ti^{W}Ea; Ti^{W}R3), while flash chromatography of the methanolic crude extract of *T. brownii* led to five fractions (Tb01, Tb02, Tb03, Tb04 and Tb05) that were similarly tested for antiplasmodial activity and cell cytotoxicity (Table 2, Fig. 3).

Table 2. Antiplasmodial activity and selectivity of fractions obtained from *T. ivorensis* and *T. brownii* aqueous and methanolic crude extracts.

Plants	Crude extract code	Solvent/Eluant	Fraction code	Antiplasmodial activity			Cell cytotoxicity		***Selectivity index			
				*IC50 ± SD (µg/mL)		RI	**CC50 ± SD (µg/mL)		SIVC		SIRC	
				*Pf*3D7	*Pf*Dd2		VC	RC	*Pf*3D7	*Pf*Dd2	*Pf*3D7	*Pf*Dd2
T. ivorensis	Ti^{W}	Methylene Chloride	Ti^{W}Chl	>100	23.71 ± 0.34	>0.23	>250	171.6 ± 0.05	–	>10	–	7.23
		Ethyl acetate	Ti^{W}Ea	0.52 ± 0.01	0.24 ± 0.06	0.46	>250	>250	>480	>1041	>480	>1041
		Aqueous	Ti^{W}R3	1.88 ± 0.16	1.46 ± 0.08	0.77	>250	>250	>132	>171	>132	>171
T. brownii	Tb^{M}	Hex:Ea (1:3)	Tb01	0.15 ± 0.00	1.73 ± 0.09	11.53	>250	>250	>1666	>144	>1666	>144
		Hex:Ea (1:1)	Tb02	>100	6.74 ± 0.07	>0.06	>250	>250	–	>37	–	>37
		Hex:Ea (3:1)	Tb03	18.30 ± 0.64	17.24 ± 0.08	0.94	>250	161.7 ± 0.02	>14	>14.50	8.83	9.37
		Hex:Ea (0:1)	Tb04	17.27 ± 0.08	25.04 ± 0.02	1.44	>250	>250	>14.47	>10	>14	>10
		MeOH:Ea (1:1)	Tb05	0.94 ± 0.00	0.95 ± 0.01	1.01	>250	>250	>265	>263	>265	>263
Reference Drugs			Artemisinin (nM)	3.38 ± 0.066	14. 47 ± 0.02	4.3						
			Chloroquine (nM)	7.55 ± 0.01	133 ± 0.02	17.61						

Inhibitors were tested against parasites in culture. *IC_{50}: Median inhibitory concentration; **Cytotoxicity of compounds was tested against normal mammalian cells CC_{50}: Median cell cytotoxic concentration; Data are mean values of triplicate experiments; ***Selectivity values represent the ratio of CC_{50} to IC_{50}; RI: IC_{50}*Pf*Dd2/IC_{50}*Pf*3D7; Art: Artemisinin; CQ: Chloroquine; Chl: Methylene chloride; MeOH: Methanol; Hex: Hexane, Ea: Ethyl acetate; Ti: *Terminalia ivorensis;* Tb: *Terminalia brownii;* W*:* Water*;* R3*:* Residue. RC: Raw Cells, VC: Vero cells, SD: Standard Deviation, SI: Selectivity Index, RI: Resistance Index.

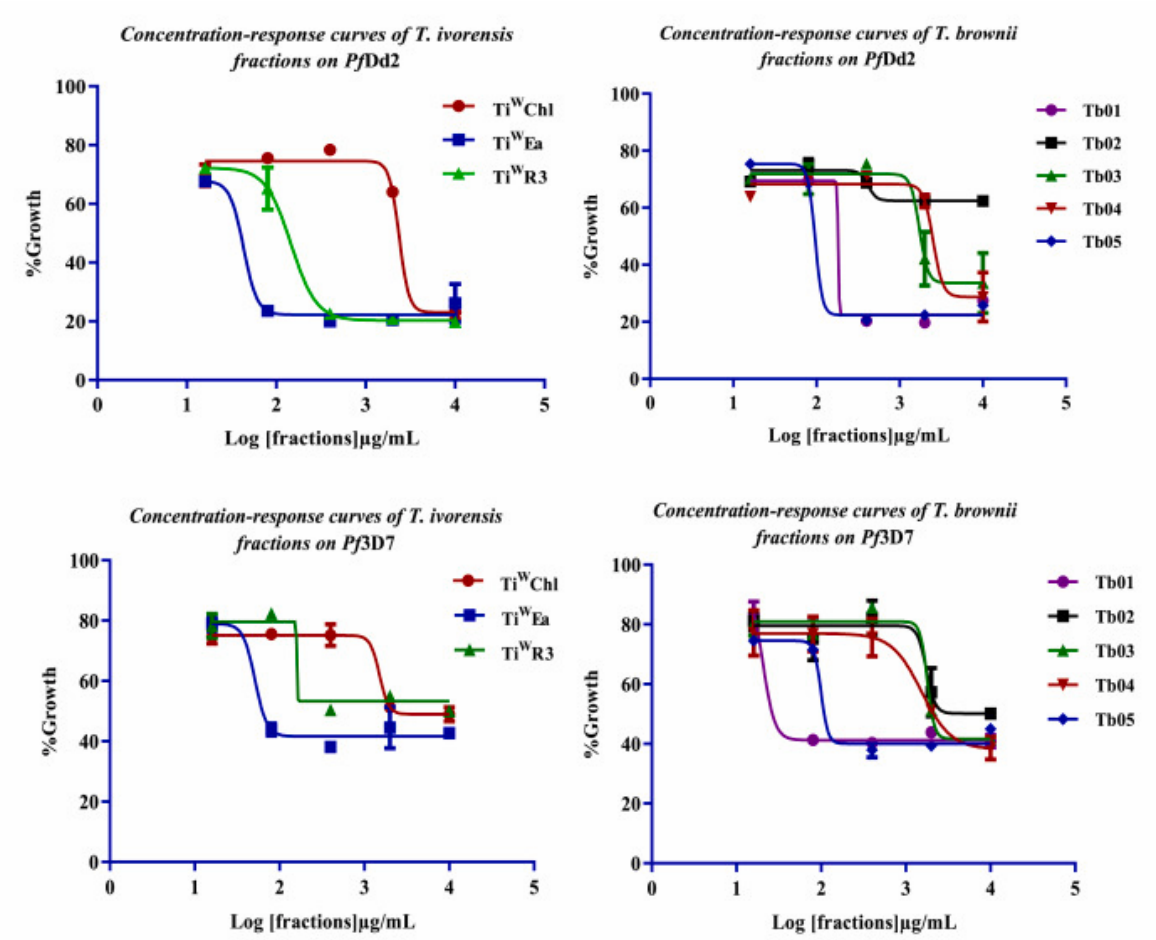


Fig. 3. Concentration–response curves of fractions from *T. ivorensis* and *T. brownii* aqueous and methanolic crude extracts on *P. falciparum* Dd2 and 3D7. Data were normalized to percent control activity and median inhibitory concentrations (IC_{50}s) calculated using Prism 5.0 software (GraphPad) with data fitted by nonlinear regression to the variable slope sigmoidal dose–response formula $y = 100/[\ 1 + 10^{(\log IC50 - x)H}]$, where *H* is the Hill coefficient or slope factor. $Ti^{W}Ea$: ethyl acetate fraction derived from the aqueous extract of *T. ivorensis*; $Ti^{W}Chl$: dichloromethane fraction derived from the aqueous extract of *T. ivorensis*; $Ti^{w}R3$: aqueous residue derived from the aqueous extract of *T. ivorensis*; Tb01: 25% Hex/Ea fraction derived from the methanol extract of *T. brownii*; Tb02: 50% Hex/Ea fraction derived from the methanol extract of *T. brownii*; Tb03: 75% Hex/Ea fraction derived from the methanol extract of *T. brownii*; Tb04: 100% Ea fraction derived from the methanol extract of *T. brownii*; Tb05: 100% MeOH fraction derived from the methanol extract of *T. brownii*.

Overall, two fractions from *T. ivorensis* [$Ti^{W}Ea$; $Ti^{W}R3$] and two from *T. brownii* [Tb01; Tb05]) exhibited highly potent inhibitory effects on both *P. falciparum* strains, with IC_{50} values ranging from 0.15 to 1.88 µg/mL and high selectivity (SI > 100). The three fractions exhibited the highest antiplasmodial potency against both strains of *P. falciparum viz.* $Ti^{W}Ea$ (IC_{50} *Pf*3D7 = 0.52 µg/mL; IC_{50} *Pf*Dd2 0.24 µg/mL), Tb01 (IC_{50} *Pf*3D7 0.15 µg/mL; IC_{50} *Pf*Dd2 1.73 µg/mL) and Tb05 (IC_{50} *Pf*3D7 0.94 µg/mL; IC_{50} *Pf*Dd2 0.95 µg/mL). These three highly potent fractions also showed high selectivity (SI > 100), serving as an indicator of their safety toward human cells and a potential source of nontoxic drug candidates for malaria control. Two other fractions, Tb03 and Tb04, displayed moderate (IC_{50} 10–20 µg/mL) to low (IC_{50} 20–40 µg/mL) antiplasmodial activity against *P. falciparum* strains. Of note, the methylene chloride fraction ($Ti^{W}Chl$) from Ti^{W} and the Hex/Ea 50% fraction (Tb02) from Tb^{M} were inactive against the chloroquine-sensitive *Pf*3D7 strain but showed moderate to

low activity against the multidrug-resistant (*Pf*Dd2) strain of *P. falciparum* (IC_{50} values of 23.71 µg/mL and 6.74 µg/mL, respectively).

3.3. Qualitative chemical profiling of extracts and fractions

Analysis of the UPLC–MS data obtained from the electrospray positive mode of the aqueous extract of *T. ivorensis* (Ti^W) and comparison to the Waters UNIFI® Scientific Information System led to the identification of two compounds, ellagic acid (**1**) and leucodelphidin (**2**) (Fig. 4, Table 3).

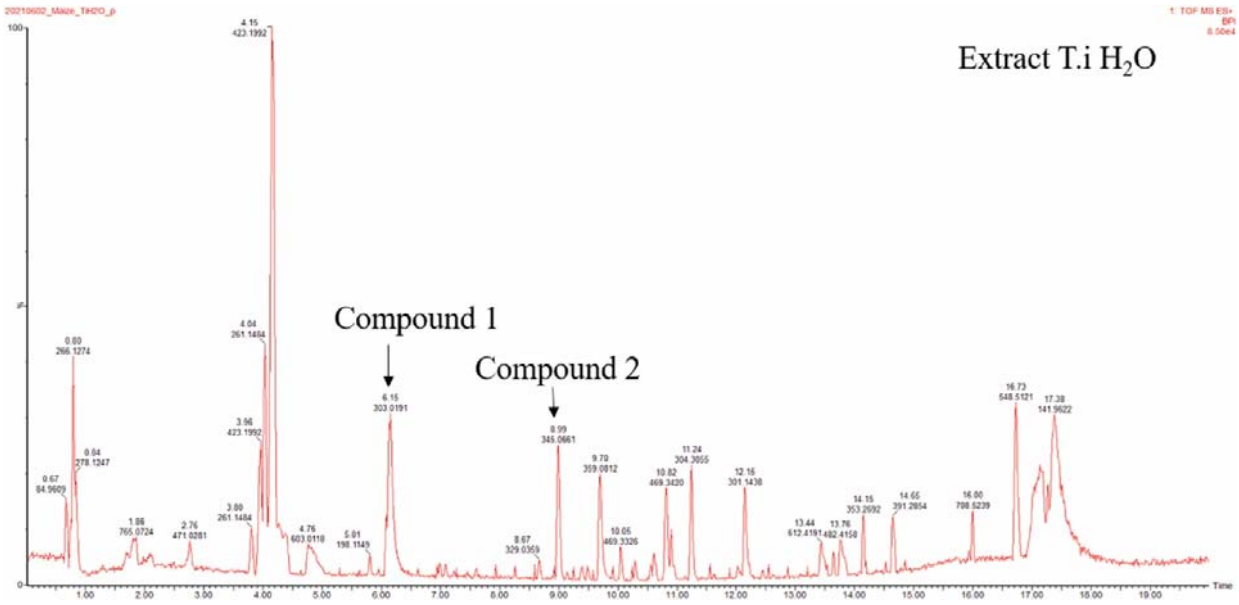


Fig. 4. UPLC–MS chemical profile (positive mode) of the crude aqueous extract of *T. ivorensis* showing the two peaks identified as chemical markers.

Table 3. Compounds identified in the crude aqueous extract of *T. ivorensis*.

Peak	Retention time	Acquired $[M+H]^+$ *m/z*	Exact mass	Formula	compound
1	6.17	303.0115	302.006270	$C_{14}H_6O_8$	Ellagic acid (**1**)
2	8.99	345.0560 $(M + Na)^+$	322.068870	$C_{15}H_{14}O_8$	Leucodelphidin[a] Leucodelphinidin[b] Gallocatechin-4-beta-ol (**2**)

[a]Most probable compound based on retention time (RT) and molecular weight (MW).
[b]Denotes synonyms.

Liquid–liquid partitioning of *T. ivorensis* crude aqueous extract resulted in fractions Ti^WChl (using dichloromethane), Ti^WEa (using ethyl acetate) and Ti^WR3 (the remaining aqueous residue). Ellagic acid (**1**) was identified in both fractions Ti^WEa and Ti^WR3, while leucodelphidin (**2**) was identified in both fractions Ti^WEa and Ti^WChl (Fig. 5).

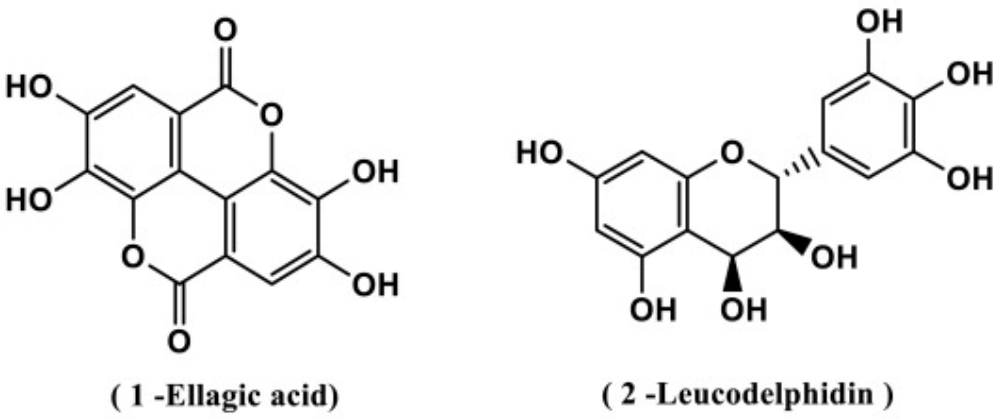
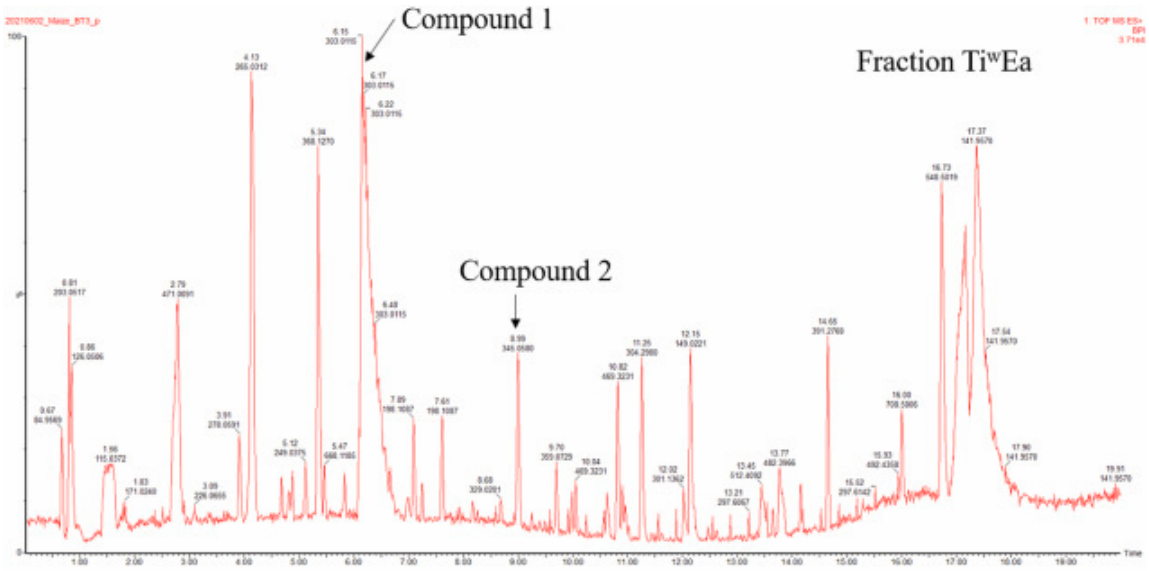
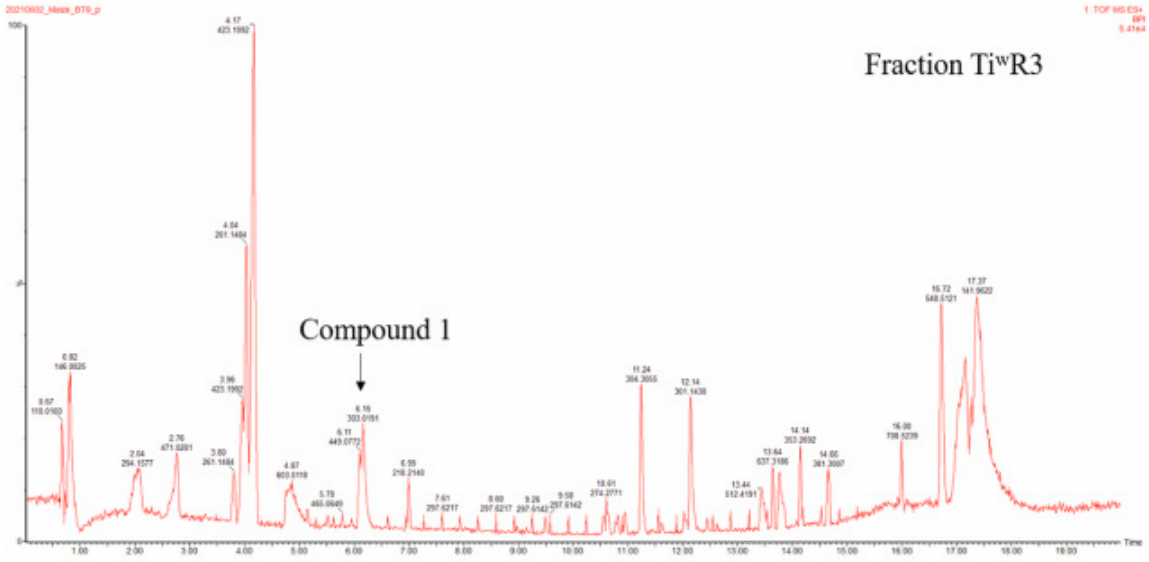


Fig. 5. Chemical structures of compounds identified in the aqueous stem bark extract of *T. ivorensis*.

Interpretation of the UPLC–MS data obtained from the electrospray positive mode of the methanolic extract of *T. brownii* and comparison to the Waters UNIFI® Scientific Information System led to the identification of five compounds, miltiodiol (**5**), leucodelphidin (**2**), papyriogenin D (**3**), dihydroactinidiolide (**4**), ellagic acid (**1**), in the methanol extract of *T. brownii* (Fig. 6, Table 4).


Compound 1
Fraction Ti^{w}Ea
Compound 2


Fraction Ti^{w}R3
Compound 1

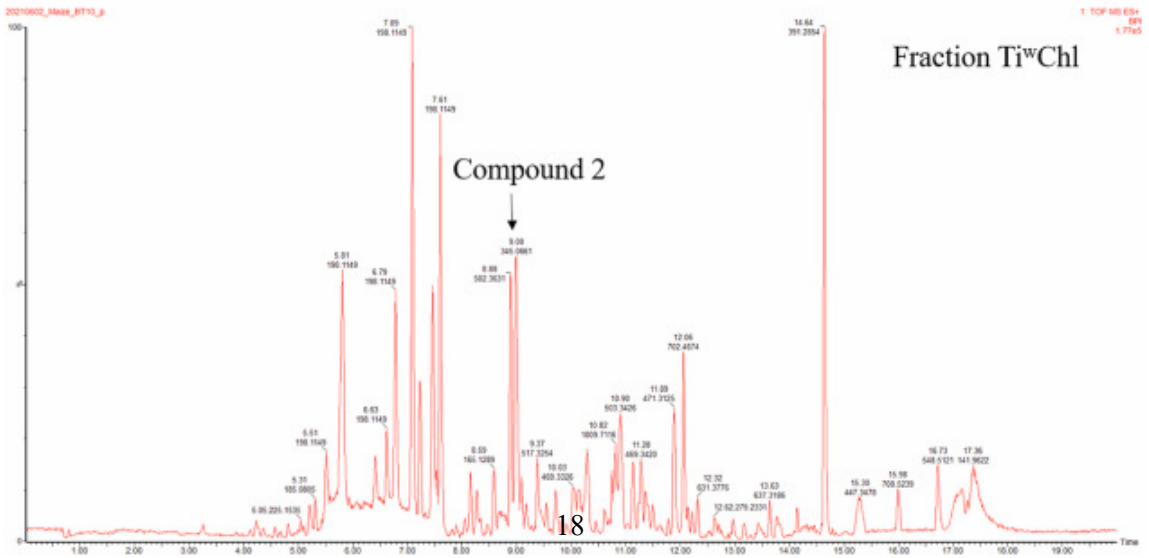

Fraction Ti^{w}Chl
Compound 2

Fig. 6. UPLC–MS chemical profiles (positive mode) of fractions $Ti^{W}Ea$, $Ti^{W}R3$ and $Ti^{W}Chl$ from the crude aqueous extract of *T. ivorensis.*

Table 4. Compounds identified in the crude methanol extract of *T. brownii*.

Peak	Retention time	Acquired [M+H]+m/z	Exact mass (*m/z*)	Molecular Formula	Compound	Reference
1	6.17	303.0191	302.006270	$C_{14}H_6O_8$	Ellagic acid (**1**)	Waters UNIFI®
2	8.34	181.1213	180.115030	$C_{11}H_{16}O_2$	Dihydroactinidiolide (**4**)	Waters UNIFI®
3	8.97	469.3309	468.323960	$C_{30}H_{44}O_4$	Papyriogenin D (**3**)	Waters UNIFI®
4	9.00	345.0580 $(M + Na)^+$	322.068870	$C_{15}H_{14}O_8$	Leucodelphidin[a] Leucodelphinidin[b], Gallocatechin-4-beta-ol[b] (**2**)	Waters UNIFI®
5	11.17	299.1610	298.156895	$C_{19}H_{22}O_3$	Miltiodiol (**5**)	Waters UNIFI®

[a]Most probable compound based on retention time (RT) and molecular weight (MW).
[b]Denotes synonyms.

Fractionation of the *T. brownii* crude methanol extract using flash column chromatography resulted in fractions Tb01, Tb02, Tb03, Tb04, and Tb05. Fig. 7 shows the LC–MS chemical profiles (electrospray positive mode) of these fractions and the compounds identified from the crude methanol extract of *T. brownii*. Ellagic acid (**1**) was identified in fraction Tb05, while dihydroactinidiolide (**4**) and miltiodiol (**5**) were identified in fraction Tb01. Leucodelphidin (**2**) was identified in both fractions Tb02 and Tb03. Papyriogenin D (**3**) was identified in both fractions Tb04 and Tb05 (see Fig. 9) (see Fig. 8).

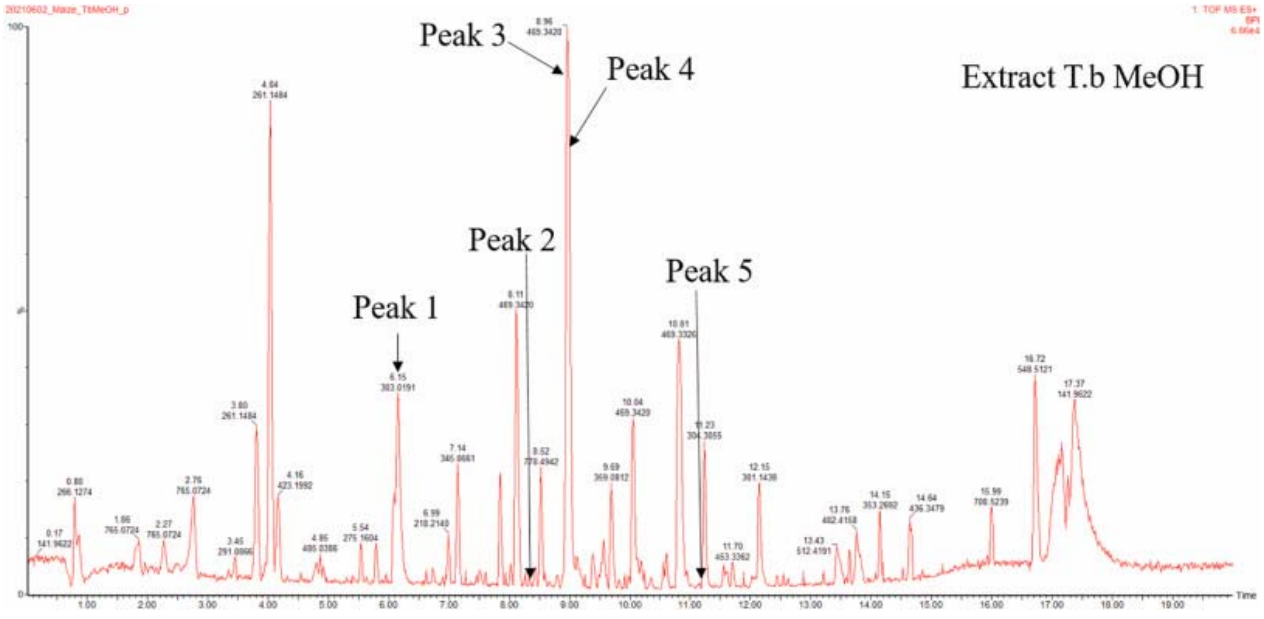


Fig. 7. UPLC–MS chemical profile (positive mode) of the crude methanol extract of *T. brownii* showing the five peaks identified as chemical markers.

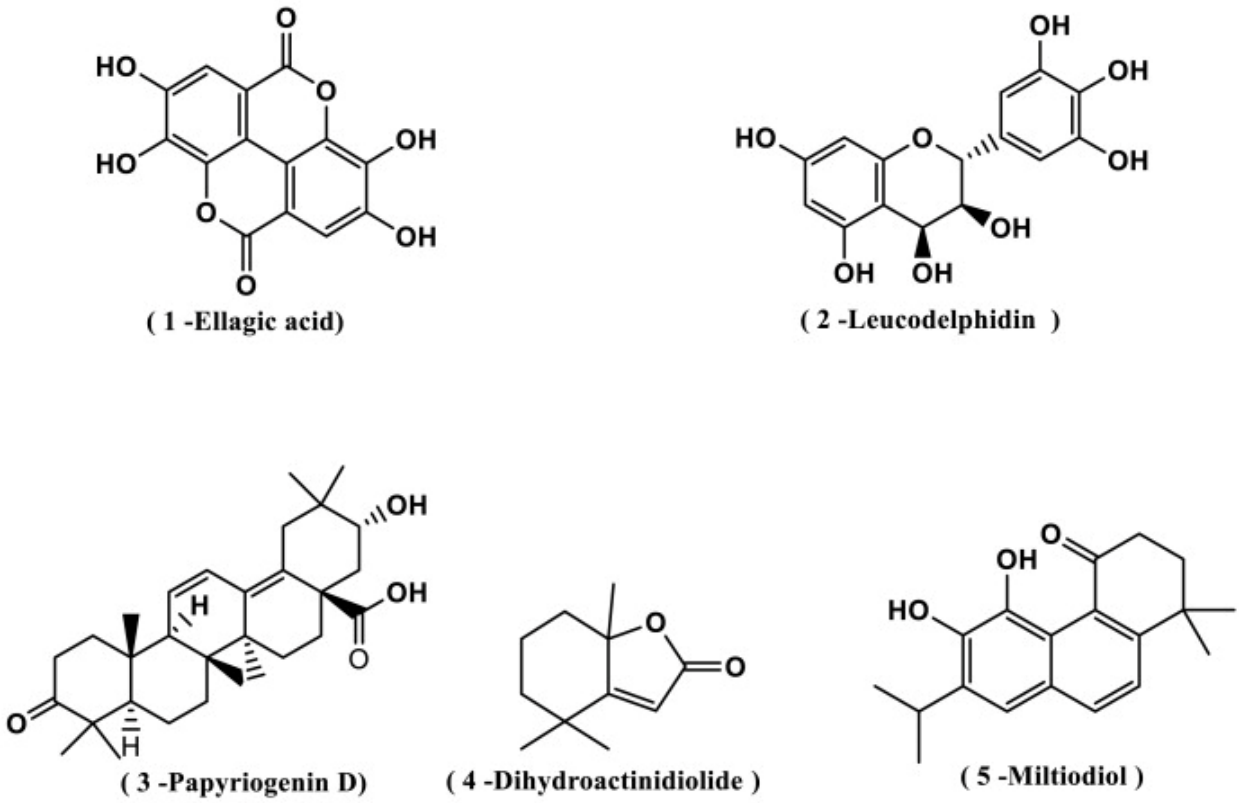


Fig. 8. Chemical structures of compounds identified in the methanolic stem bark extract of *T. brownii*.

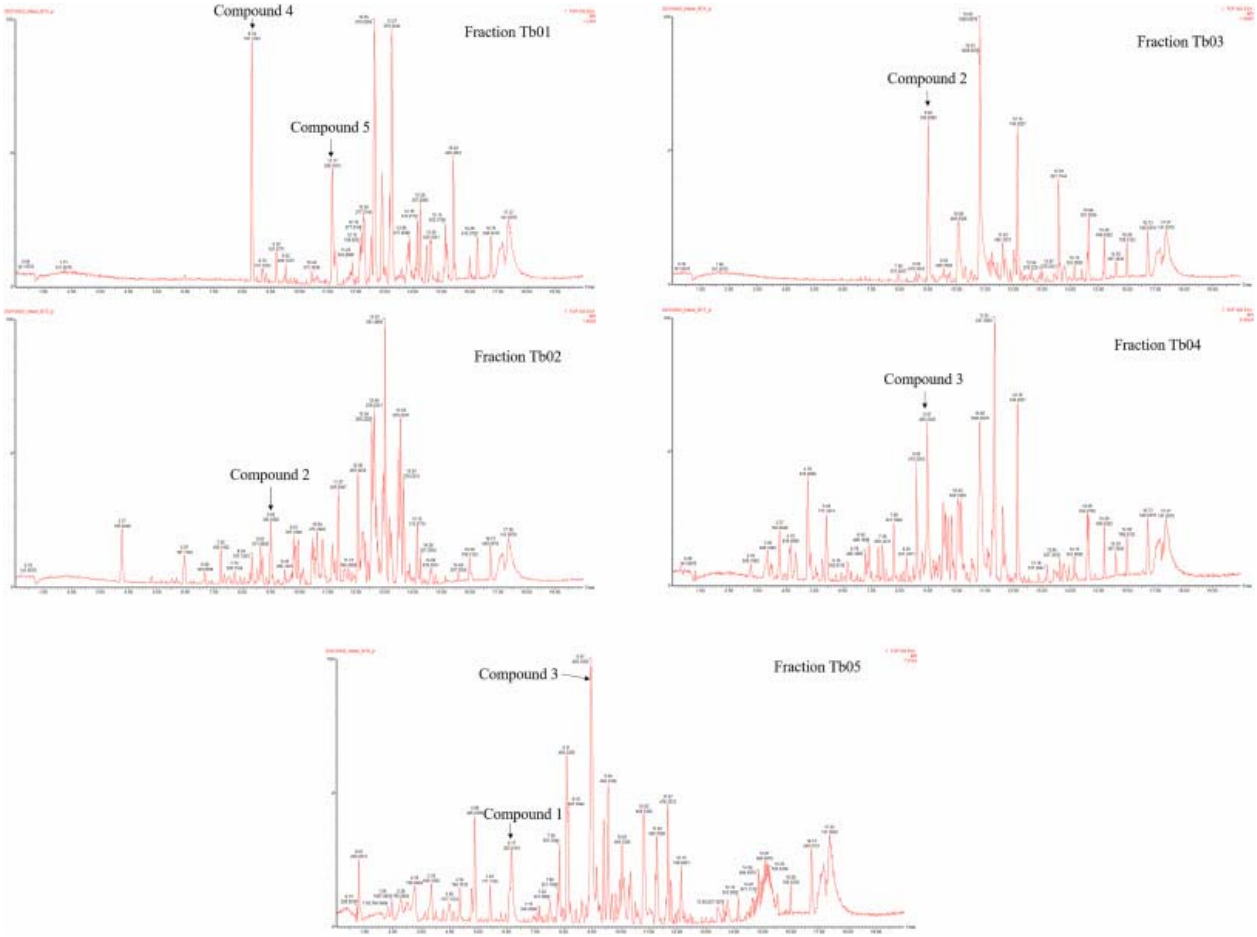


Fig. 9. UPLC–MS chemical profiles (positive mode) of fractions Tb01, Tb02, Tb03, Tb04 and Tb05 and the crude methanol extract of *T. brownii.*

Ellagic acid (**1**) and leucodelphidin (**2**) were identified from the extracts of both *Terminalia* species. Since ellagic acid (**1**) appeared as the most intense peak in fraction Ti^{W}Ea and was common to many of the active fractions, this pointed to it being a bioactive compound. The compound standard was purchased and analyzed by UPLC MS and bioassayed, resulting in significant activity against both multidrug-resistant (*Pf*Dd2; IC_{50} = 0.59 ± 0.08 μM) and chloroquine-sensitive (*Pf*3D7; IC_{50} = 2.68 ± 0.08 μM) strains of *P. falciparum.* UPLC-QTOF-MS analysis of the purchased analytical standard of ellagic acid and interpretation of the data obtained in the positive mode confirmed the presence of the compound in the extracts and bioactive fractions (Table 5).

Table 5. Comparison of UPLC–MS data of ellagic acid (**1**) from analysis of the purchased standard and the crude aqueous extract of *T. ivorensis*.

Sample	Retention time	Acquired [M+H]+*m/z*	Molecular Formula	MS/MS data (fragments)
Ellagic acid in *T. ivorensis* aqueous extract	6.15	303.0191	$C_{14}H_6O_8$	257.0141 201.0214 173.0293
Standard ellagic acid	6.14	303.0182	$C_{14}H_6O_8$	257.0129 201.0199 173.0278

4. Discussion

Plant Kingdom stands as an infinite resource for discovery of novel chemotypes and pharmacophores for amplification into efficacious drugs for a multitude of disease indications. As such, until recently, many novel pharmacologically active compounds with many blockbuster drugs were derived directly or indirectly from plants. Even at the dawn of the 21st century, 11% of the 252 drugs considered basic and essential by the WHO were exclusively of flowering plant origin (Veeresham, 2012). For the purpose of drug discovery, profiling the chemical composition of plant extracts is of utmost importance and fundamentally orients the unveiling of new chemical scaffolds with the potential to provide a novel and diverse range of promising drugs to control many diseases, including malaria. Two medicinal plants from Cameroon, *T. ivorensis* and *T. brownii,* used traditionally for treating malaria, were investigated for their activity against chloroquine-sensitive (3D7) and multidrug-resistant (Dd2) strains of *P. falciparum*. Extracts obtained from the two plants displayed potent antiplasmodial activity. Further bioassay-guided fractionation of selected extracts (Ti^w and Tb^M) led to the identification of two highly potent fractions, Ti^wEa and Tb05, with IC_{50} values < 1 µg/mL and SI > 250 for both chloroquine-sensitive and multidrug-resistant strains of *P. falciparum,* respectively. Chemical profiling using UPLC–MS led to the identification of ellagic acid and leucodelphidin from the aqueous extract of *T. ivorensis* and dihydroactinidiolide, miltiodiol, leucodelphididin, papyriogenin D, and ellagic acid from the methanol extract of *T. brownii*. Of note, ellagic acid was previously reported to selectively display significant antiplasmodial activity with IC_{50} values ranging from 105 to 330 nM regardless of the chemosensitivity of the *P. falciparum* strain tested and to exhibit significant antimalarial efficacy (suppressive, curative and prophylactic) in *Plamodium vinckei*-infected mice (Soh et al., 2008). The preferential targets of action of this compound were reported to be the trophozoite and schizont stages of the *Plasmodium* intraerythrocytic life cycle (Soh et al., 2008). Within the scope of this work, the reported activity profile of ellagic acid was corroborated through the investigation of a commercially sourced compound that displayed IC_{50} values of 0.59 µM and 2.68 µM against *Pf*Dd2 and *Pf*3D7. This finding further validated the active input of ellagic acid to the displayed antiplasmodial activity of extracts and fractions from both *Terminalia* species. Similarly, Machumi et al. (2013) previously reported a phytochemical investigation of the ethyl acetate-soluble fraction from the stem bark of *T. brownii* collected in Kenya, leading to the isolation of a new oleanane-type triterpenoid, along with seven known triterpenoids, seven ellagic acid derivatives, and 3-*O*-β-D-glucopyranosyl-β-sitosterol. Among these natural products, two ellagic acid derivatives bearing a galloyl group (4-*O*-(3",4"-di-*O*-galloyl-α-L-rhamnopyranosyl and 23-galloylarjunic acid) were found to be moderately active against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *P. falciparum* with IC_{50} values ranging from 2.8 to 4.7 µg/ml. Of note, the compounds identified in this study from the methanolic extract of *T.*

brownii stem bark (ellagic acid, leucodelphidin, dihydroactinidiolide, miltiodiol and papyriogenin D) were not reported by Machumi et al. (2013) while investigating the ethyl acetate extract. On the other hand, the biological mechanisms of action of ellagic acid were previously reported to include DNA topoisomerase inhibition, the induction of cell cycle arrest, and the activation of apoptotic pathways (Constantinou et al., 1995). In addition, its strong antiplasmodial activity has been attributed to the inhibition of Plasmepsin II, the reduction of glutathione content inside the *Plasmodium* parasite and an impairment of beta-hematin formation (Constantinou et al., 1995; Dell'Agli et al., 2003; Sturm et al., 2009). From this rationale, we can hypothesize that part of the antiplasmodial activity displayed by extracts and fractions from both *Terminalia* spp. is attributable to ellagic acid, which elicits bioactivity through the inhibition of molecular targets of critical importance to *Plasmodium* survival.

Activity-directed fractionation of potent extracts from both plants led to more potent fractions in which active principles may have been concentrated, with potential synergistic interaction against the parasites as previously alluded (Nwodo et al., 2010; Rasoanaivo et al., 2011)**.** Activity data generated from this investigation confirmed the previously reported antiplasmodial potential and selectivity of extracts from *Terminalia* species. For instance, Wande and Babatunde (2018) reported the antiplasmodial IC_{50} of *T. ivorensis* leaf methanolic extract to be 2.58 mg/mL using the β-hematin synthesis inhibitory assay. This finding further suggests that antiplasmodial metabolites are secreted from the metabolic performance in different parts of this plant. Likewise, using the thin blood film approach, Annan et al. (2012) reported the antiplasmodial activity of the ethanolic extract from the stem bark of *T. ivorensis* against *P. falciparum* 3D7 with an IC_{50} of 6.94 µg/mL, suggesting that antiplasmodial metabolites are diversely and readily extracted from *T. ivorensis* by solvents of varying relative polarities (ethyl-acetate-0.228, ethanol-0.654, methanol-0.762, water-0.991-1.000). Of note, the values for relative polarity are normalized from measurements of solvent shifts of absorption spectra (Reichardt, 2003). Notably, the ethanolic extract from the stem bark of the Cameroonian *T. ivorensis* investigated in the present study exerted 4-8-fold more potency against *P. falciparum* 3D7 and Dd2 strains than the extract from the Ghanaian species against *P. falciparum* 3D7. This discrepancy in activities further indicates that the origin of plants might play a critical role in the quality of bioactive secondary metabolites present in the crude extract (Demain and Fang, 2000). Moreover, the 4-8-fold shift in the activity of the *T. ivorensis* extract between this study and the report by Annan et al. (2012) denotes the impact of the adopted screening procedures that undoubtedly influence the displayed activity parameters. Indeed, Annan et al. (2012) used the microscopic technique to assess antiplasmodial activity, while the *Pf*LDH-based assay was used in the present study. From a wider perspective, plant extracts from the *Terminalia* genus have consistently shown antiplasmodial activity. Recently, Mbouna et al. (2018) reported the antiplasmodial activity of the stem bark and leaf extracts of *T. mantaly* and *T. superba* with potent activities (IC_{50}: 0.26–1.26 µg/mL) and selectivity (SI > 158) against resistant INDO and sensitive 3D7 strains of *P. falciparum*. Additionally, water decoction extracts of leaf of *T. catappa* and leaf and bark of *T. mantaly* showed antiplasmodial activities with IC_{50} values of 6.41/8.10 µg/mL, 2.49/1.90 µg/mL and 3.70/2.80 µg/mL, respectively, against the 3D7/INDO strains of *P. falciparum* (Mbouna et al., 2018)**.** Additionally, reports indicated that *T. avicennioides* leaf and stem bark aqueous, methanol, butanol, ethyl acetate and dichloromethane extracts showed antiplasmodial activity with IC_{50} values ranging from 1.60 to 7.40 µg/mL against the *P. falciparum* K1 strain (Sanon et al., 2013; Ouattara et al., 2014). In addition to ellagic acid identified as the main constituent from the extracts of *T. ivorensis* and *T. brownii*, additional phytoconstituents were detected, notably leucodelphidin (flavonoid), dihydroactinidiolide

(benzofuran) and two terpenoids (miltiodiol and papyriogenin D). We could tentatively conclude that the latter secondary metabolites add their potential to ellagic acid to elicit stronger antiplasmodial activity. Of note, compounds belonging to similar phytochemical classes were previously implicated in the antiplasmodial activity of plant extracts (Zofou et al., 2012; Kamkumo et al., 2012; Malterud, 2017). In that regard, flavonoids have been reported to exert pronounced to moderate activity against the asexual blood stage of *P. falciparum* strains and clinical isolates (Ganesh et al., 2012). Previous reports also indicated that flavonoids can interface and prohibit the action of *P. falciparum* cysteine protease (falcipain), a vital enzyme involved in hemoglobin digestion inside the acidic food vacuole of the intraerythrocytic parasite. They have been reported to hinder plasmodial hemozoin formation in culture, leading to the death of *P. falciparum* parasites (Sinha et al., 2019). In addition, terpenes such as the antimalarial drug artemisinin (a sesquiterpene lactone) are reputed to inhibit the biosynthesis of both dolichol and the isoprene side chain of ubiquinone and the isoprenylation of proteins in the intraerythrocytic stages of *P. falciparum,* leading to the death of the parasite (Goulart et al., 2004). Previous investigations also showed that terpenes are activated by heme to generate free radicals, which in turn damage proteins required for parasite survival (Tilley et al., 2016; Wang et al., 2015).

5. Conclusion

The information generated from this first-in-kind bioguided study of *T. ivorensis* and *T. brownii* extracts provides an extent of scientific evidence to support their traditional use in the treatment of malaria. Based on *in vitro* data, prioritized extracts and fractions (IC_{50} < 1 μg/mL) from the two plants are highly selective at concentrations <250 μg/mL. However, *in vivo* and clinical safety data are required to justify human consumption. Ellagic acid was identified in the extracts and was subsequently validated as an active antiplasmodial compound in extracts of both *Terminalia* spp. This study is an initial step toward a full characterization of the antiplasmodial potential of the two promising *Terminalia* extracts. The identified potent and selective fractions might be formulated as antimalarial phytodrugs, or further fractionated to afford pure entities bearing the antiplasmodial potency.

CRediT authorship contribution statement

Mariscal Brice Tchatat Tali: Investigation, Software, Visualization, Validation, Writing – original draft, Writing – review & editing. **Darline Dize:** Investigation, Methodology. **Steven Collins Njonte Wouamba:** Investigation, Methodology. **Patrick Valere Tsouh Fokou:** Visualization, Supervision, Writing – review & editing. **Rodrigue Keumoe:** Investigation. **Cyrille Njanpa Ngansop:** Investigation. **Michelle Sidoine Nguembou Njionhou:** Investigation. **Cedric Derick Jiatsa Mbouna:** Investigation, Data curation. **Lauve Rachel Yamthe Tchokouaha:** Visualization, Supervision, Writing – review & editing. **Vinesh Maharaj:** Investigation, Data curation, Supervision, Writing – review & editing. **Ndivhuwo Kevin Khorommbi:** Investigation, Writing – review & editing. **Dashnie Naidoo-Maharaj:** Investigation, Writing – review & editing. **Jean Claude Tchouankeu:** Conceptualization, Supervision, Writing – review & editing. **Fabrice Fekam Boyom:** Funding acquisition, Conceptualization, Validation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest**.**

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Data availability

Data will be made available on request.

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