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Efficacy of medicinal plants and their derived biomolecules against apicomplexan pathogen

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Abstract

Background: Many apicomplexan pathogens pose significant threats to humans and domestic animals, with the lack of effective drugs and drug resistance representing major challenges in disease management. To address this, the search for new and potent antimalarial drugs is crucial. Plant-based formulations offer a promising alternative for such drug development. Here, we evaluated the *in vitro* antiplasmodial activity of nine plant extracts, traditionally used to treat fever-like symptoms in Bangladesh.

Methods: We assessed the antimalarial activity of plant extracts by using the *Plasmodium falciparum* 3D7 growth inhibition assay, an invasion assay, and a cytotoxicity assay.

Results: Of the nine plants studied, ethanolic and methanolic leaf extracts of *Ficus hispida*, *Streblus asper*, and *Boerhavia repens* exhibited high antiplasmodial activity, with IC_{50} values of 9.31, 4.13, 9.63 µg/ml (ethanolic) and 15.58, 6.63, 7.58 µg/ml (methanolic), respectively, and minimal toxicity (cell viability >80%). *Clerodendrum viscosum* displayed antiplasmodial effects with IC_{50} values of 42.43 µg/ml (ethanolic) and 27.01 µg/ml (methanolic). *Adhatoda vasica*, *Mussaenda corymbosa*, and *Amaranthus spinosus* ethanolic extracts showed antimalarial effects with IC_{50} values of 59.59 µg/ml, 57.09 µg/ml, and 64.14 µg/ml, respectively. However, methanolic extracts of *Adhatoda vasica* and *Amaranthus spinosus* had IC_{50} values >100 µg/ml. The ethanolic and methanolic extracts of *Adhatoda vasica*, *Amaranthus spinosus*, *Ficus hispida*, *Streblus asper*, and *Boerhavia repens* significantly reduced parasitemia by inhibiting invasion into erythrocytes.

Conclusions: This study highlights the robust antimalarial activity and low cytotoxicity of leaf extracts of *Ficus hispida*, *Streblus asper*, and *Boerhavia repens*, indicating the presence of antimalarial compounds that warrant further investigation.

Background

Apicomplexans have a considerable impact on public health globally, causing diseases such as toxoplasmosis, cryptosporidiosis, babesiosis, and malaria. Toxoplasmosis and cryptosporidiosis are zoonotic diseases that seriously threaten human life (1–3). Apicomplexans have great adaptability to the environment, as evidenced by their complex evolutionary genome (4, 5). It has been reported that this highly complex genomic structured pathogen becomes resistant to commercially available medications within about 15 years of their clinical use. For example, *Plasmodium* has developed resistance to artemisinin-based synergistic applications (6, 7). Almost all the currently approved anti-apicomplaxan drugs were discovered either through chemical modification of natural products or through large-scale screening of plant-derived biomolecules (8).

Malaria is one of the most life-threatening infectious diseases (9, 10). It is attributed to the obligatory intracellular protozoan parasites within the *Plasmodium* genus (11). The causative agents encompass a variety of *Plasmodium* protozoa, namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi* (12, 13). *Plasmodium falciparum* causes severe

and life-threatening malaria, whereas *P. vivax* is associated with malaria relapse(9, 14). The global malaria burden statistics for 2021––with an estimated 247 million cases and 619,000 deaths––underscore the ongoing significance of malaria as a major public health concern (9). The most infected individuals are pregnant women and children (9, 10). Since ancient times, anti-pathogenic activity has been found in various medicinal plants. Plants are a major source of herbal medicines (15). People utilize their ethnobotanical knowledge to develop remedies and uses for medicinal plants (11). The plant-derived natural compound quinine used as a remedy from malaria and malaria-like symptoms was first isolate in 1820 almost 60 years before the discovery of the causal agents of malaria (16). Quinine, derived from the bark of South American cinchona trees, serves as the key ingredient in a centuries-old traditional antimalarial remedy (17).

Artemisinin was discovered through the Chinese military Project 523, which focused on screening traditional medicines and folk remedies for antimalarial activity using the *P. berghei* model (8). This active phytocompound was isolated in 1971, by Professor and Nobel laureate Youyou Tu, from the *Artemisia annua* plant, a common ingredient in ancient Chinese antipyretic plant extracts. Chinese herbalists have used extracts of the Qinghao plant *Artemisia annua*, traditionally named as sweet wormwood, for malaria treatment for over 1,500 years (15).

Drug resistance to available malaria drugs creates challenges for those treating this devastating disease (6, 7). Resistance has been observed against the new drug cipergamin during clinical treatment (18). Therefore, the ongoing search for new antimalarial agents is imperative (19). Medicinal plants as sources of antimalarial treatment would be beneficial because such drugs would be cheaper to produce, more cost effective for patients, and easily accessible.

Many indigenous communities and tribes have high incidences of malaria, because they live within or near mosquito-infested forest regions (20, 21). For centuries, tribal medicinal practitioners have treated malaria or malaria-like symptoms with various plant-based formulations like *Clerodendrum viscosum, Amaranthus spinosus, mussaenda corymbosa*, and *Streblus asper* (21). Here we evaluated the *in vitro* antiplasmodial activities of ethanolic and methanolic leaf extracts from nine traditional plants in Bangladesh against *Plasmodium falciparum* 3D7.

Materials and Methods

Collection of plant materials and identification

Plant materials were collected from Rajshahi, Bangladesh (24.375508258708045, 88.60334324068714). Plant material identification was verified by the plant biologists in the Department of Botany at the University of Rajshahi, Bangladesh.

Preparation of plant materials

After collection of plant samples, the leaf parts were carefully separated. The fresh plant parts were washed thoroughly 3–4 times with running tap water and then finally with distilled water. Subsequently, the plant

materials were shade-dried at room temperature for 20–25 days. The dried plant materials were then ground into a coarse powder by using a grinder machine and stored in an airtight container at room temperature for subsequent analyses. A portion of the powdered material was specifically used for crude extraction.

Preparation of plant extracts

About 20 grams of dried sample powder for each specimen was combined at a weight/volume (w/v) ratio of 1:10 with pure ethanol and methanol as solvents. The mixture was filtered through cotton cloth and subsequently through Whatman no. 1 filter paper. The extraction process involved shaking for 48 h in a shaker machine. The resulting extracts were concentrated to dryness using a water bath set at 35°C and then stored in sterile bottles at 4–8°C for subsequent analyses. The extraction percentage was calculated using the formula:

%Extraction = weight of the extract (g) / weight of the plant material (g) ×100

Parasites culture and maintenance

The *Plasmodium falciparum* 3D7 strain was cultured with blood stage parasites for this study, incorporating modifications to a previously established method (22, 23). In brief, parasites were grown in human red blood cells (RBCs) and maintained in culture medium consisting RPMI 1640 (Sigma-Aldrich), 25 mM HEPES, 100 µM hypoxanthine (Wako), 12.5 µg/ml gentamycin (Sigma-Aldrich), 0.5% (w/v) Albumax II (Invitrogen), and 62.5 µg/ml $NaHCO_3$ (Wako). The culture was maintained at 37°C, 5% O_2, and 5% CO_2, with daily medium changes. Parasite growth and development were monitored through Giemsa staining and microscopic examination as needed. Maintaining optimal conditions for the *Plasmodium falciparum* 3D7 culture involved keeping hematocrit levels at approximately 5% and parasitemia at 10% as maximum values. Initial synchronization was achieved by subjecting early ring stage parasites to treatment with 5% sorbitol, which was filter-sterilized through a 0.45-µm Millipore filter. To obtain highly synchronized mature-stage parasites, gradient centrifugation using 40–70% Percoll (GE Healthcare) was conducted.

In vitro 293T cell culture

Human embryonic kidney (293T) cells were cultured was previously described with some modification (24). Briefly, 293T cells were grown in culture dishes 100 X 20 (Thermo Scientific, Nunclo delta surface) containing DMEM (5 ml), (Nacalai Tesque, Japan), 10% fetal bovine serum (FBS) (Gibco, USA), l-glutamine, 50 I.U/ml penicillin–50 µg/ml streptomycin, and 62.5 µg/ml $NaHCO_3$ (Wako) at 37°C, with 5% O_2 and 5% CO_2. Cell growth was examined under the microscope. When cell confluency reached 70–80%, cells attached to the culture dishes were trypsinized (Gibco, USA) for subculture. Cells were counted by using the cell counting slide Formula, $(A + B + C + D)/4 \times 10^4$ cells/ml medium. Cells were sub-cultured every 4–5 days (Thermo Scientific, Nunclon Delta surface).

Growth inhibition assay of Plasmodium falciparum

Growth inhibition assays were conducted as previously described (25). In brief, ring-stage parasites were synchronized using D-sorbitol or Percoll (GE Healthcare) gradient (70–40%) centrifugation. Subsequently, the parasites were allowed to invade fresh erythrocytes for 5 h, followed by a 5% filter-sterilized D-sorbitol treatment. After a 24-h incubation, growth inhibition assays were conducted when the parasites were at the trophozoite stage. Approximately 0.3% parasitemia and 1% hematocrit were prepared by adding uninfected human red blood cells (uRBCs). Ethanolic and methanolic leaf extracts were dissolved in distilled water and filter-sterilized through a 0.45-µm Millipore filter to make a stock solution of 20 mg/ml. Working concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.562 µg/ml were prepared. Subsequently, 147 µl of the parasite medium was seeded into each well of a microtiter plate. To achieve a total volume of 150 µl per well, 3 µl of the extracts was added. Ten micromolar artemisinin was used as a positive control, and wells with the same parasite culture medium but without drugs served as negative controls. The microtiter plates were incubated at 37°C with 5% O_2 and 5% CO_2. After 2 days of incubation, an additional 50 µl of culture medium was added to each well. The plates were then again incubated at 37°C with 5% O_2 and 5% CO_2 for another 2 days. After 4 days of incubation, when the parasites were in trophozoite or schizont stages, parasitemia was observed under an optical microscope. The following formula was used to determine the GIRs (growth inhibitory rate) (25): growth inhibitory rate (%) = { 1− [(parasitemia of the sample) − (parasitemia of the positive control)]/ [(parasitemia of the negative control) −(parasitemia of the positive control)]} ×100. The half maximal (50%) inhibitory concentration (IC_{50}) values were calculated by using the following Eq. (26):

IC_{50} = 10^ [log(A/B) *(50-C)/(D-C) + log(B)],

where

A is the minimum concentration at which the inhibition rate exceeds 50%,

B is the maximum concentration at which the inhibition rate exceeds 50%,

C is the inhibition rate with B,

D is the inhibition rate with A,

Invasion inhibition assay of Plasmodium falciparum

For the invasion assays, we employed the Percoll-Sorbitol method to isolate highly synchronized schizont-infected erythrocytes, following established protocols (11, 25, 27). In brief, parasites cultured in 10-ml flasks were centrifuged at 2,000 rpm for 5 minutes in 15-ml tubes at room temperature. The supernatant was aspirated, and a 50% hematocrit was prepared using packed erythrocytes. The solution was layered onto a gradient of 70–40% Percoll-Sorbitol solution in 15-ml tubes. The tubes were then centrifuged at 10,000 rpm for 15 minutes at room temperature. A grayish band of mature schizonts was observed at the 40/70 gradient interface, which was carefully collected using a 1-ml pipette into 15-ml tubes. Incomplete medium was added dropwise to the mature schizonts with continuous shaking. Approximately 10 ml of incomplete medium was added, and the cells were washed twice by centrifuging at 2,000 rpm for 5 minutes at room

temperature. Giemsa-stained smears were examined under a microscope to assess the purity of the isolate. The parasite number was counted by using a hemocytometer and the formula: $(A + B + C + D)/4 \times 10^4$ cells/ml medium. Approximately 1% hematocrit and 2% parasitemia were prepared with fresh erythrocytes. Culture medium (150 µl) containing the IC_{50} concentration of the plant extracts was transferred into 96-well flat bottom plates. The mature parasites with fresh RBCs were then incubated for 24 h at 37°C, with 5% CO_2 and 5% O_2.

Cytotoxicity assay

For cytotoxicity assays, Cell Count Reagent SF (Nacalai Tesque, Japan) was utilized. The 293T cells were cultured in 96-well microplates; about 5,000 cells were added to each well containing 100 µl of medium. The cells were allowed to grow to 70% confluency. The cells were incubated for 96 h with varying concentrations of extracts (1000, 100, 10, 1, 10^{-1} µg/ml). Following the 96-h incubation, 10 µl of Cell Count Reagent SF solution was added to each well and allowed to incubate for an additional 1–4 hours. Subsequently, the absorbance at 450 nm was quantified utilizing a microplate reader (Corona Electric, Ibaraki, Japan). Cell viability (%) was calculated using the following formula (25): cell viability (%) = [(absorbance at 450 nm of treated group)/(absorbance at 450 nm of control group)]×100.

Selectivity index

The selectivity index (SI) was used to compare the toxicity of extracts against human cells (i.e., 293T cells) and the *Plasmodium falciparum* parasite. We estimated the SI of *in vitro* toxicity for each extract as follows (25):

SI (%) = [(IC_{50} of 293T cell)/ (IC_{50} of *P. falciparum*)] ×100

Statistical analysis

The data, derived from three biological replications of the experiments, are graphically illustrated in the .XL file. The data are presented as mean values ± standard deviation (SD) from three biological replicates of the experiments. Statistical significance was determined using a paired Student t-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; compared with the negative control.

Results

Extraction yield

The extraction percentage was calculated by using the following formula (28):

%Extraction yield = weight of the extract (g)/weight of the plant material (g) ×100

Following the drying process, yields were obtained from the ethanolic and methanolic crude extract of leaves from all nine plant species Table 1.

Table 1
Extraction yields of the methanolic and ethanolic crude leaf extracts of nine medicinal plants

No.	Family	Scientific name	Local name in Bangladesh	%Extraction	
				Methanol	Ethanol
1.	Moraceae	*Ficus hispida*	Dumur	6.9	6.3
2.	Moraceae	*Streblus asper*	Sheora	6.7	6.35
3.	Nyctaginaceae	*Boerhavia repens*	Punarnava	7.15	5.6
4.	Clerodendaceae	*Clerodendrum viscosum*	Ghetu, bait	8.05	6.6
5.	Acanthaceae	*Adhatoda vasica*	Bashok	7.6	6.95
6.	Amaranthacae	*Amaranthus spinosus*	Kantanotey, Kantakhuira	6.35	5.45
7.	Rutaceae	*Aegle marmelos*	Bel	6.7	5.95
8.	Fabaceae	*Tamarindus indica*	Tetul	8.85	6.05
9.	Rubiaceae	*Mussaenda corymbosa*	Mok-ae	8.3	7.1
% Extraction = weight of the extract (g)/weight of the plant material (g) ×100					

In vitro growth inhibition

The ethanolic and methanolic extracts from the leaves of nine plant species from different families were tested against the CQ-sensitive *P. falciparum* 3D7 strain *in vitro*. Of the nine plant species, extracts from three, *Ficus hispida*, *Streblus asper*, and *Boerhavia repens*, exhibited strong antiplasmodium activity at the highest concentration of 25 µg/ml (Figs. 1 and 2). *Clerodendrum viscosum, Adhatoda vasica*, *Amaranthus spinosus*, and *Mussaenda corymbosa*, exhibited strong antiplasmodium activity at the highest concentration of 400 µg/ml (Figs. 3 and 4). The IC_{50} values, 50% cytotoxicity concentrations (CC_{50}) values, and sensitivity index (SI) of each extract are presented in Table 2. Ethanolic and methanolic leaf extracts of *Ficus hispida*, *Streblus asper* and *Boerhavia repens* demonstrated high antiplasmodial activity, with IC_{50} values of < 10 µg/ml (ethanolic: 9.31, 4.13, 9.63 µg/ml; methanolic: 6.63, 7.58 µg/ml), respectively. The ethanolic and methanolic extracts of *Clerodendrum viscosum*, along with the methanolic crude leaf extract of *Ficus hispida*, exhibited moderate activity, with IC_{50} values ranging from 10 to 50 µg/ml (42.43 µg/ml, 27.01 µg/ml, and 15.58 µg/ml, respectively). The ethanolic extracts of *Adhatoda vasica*, *Amaranthus spinosus*, and *Mussaenda corymbosa* exhibited mild activity, showing IC_{50} values ranging from 50 to 100 µg/ml (59.59 µg/ml, 64.14 µg/ml and 57.09 µg/ml), respectively. The methanolic leaf extracts of *Adhatoda vasica* and *Amaranthus spinosus* demonstrated antiplasmodial activity with IC_{50} values of 291.87 µg/ml and 135.19 µg/ml, respectively. Ethanolic and methanolic leaf extracts of *Ficus hispida*, *Streblus asper*, and *Boerhavia repens* showed higher antiplasmodial activity than the other plants. Of note, the ethanolic extracts of most plants exhibited higher activity than the corresponding methanolic extracts, except for the

leaves of *Boerhavia repens* and *Clerodendrum viscosum* (Table 2). The shapes of the food vacuoles of the schizonts with the extracts looked normal and different from those with cysteine inhibitors (Fig. 5).

Table 2
In vitro antiplasmodial activity and cytotoxicity of ethanolic and methanolic leaf extracts of plants used to treat malaria and malaria-like symptoms in Bangladesh

No.	Family	Plant species	Ethanolic extract			Methanolic extract		
			IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI
1	Moraceae	*Ficus hispida*	9.31	96.15	10.33	15.58	> 100	> 6.418
2	Moraceae	*Streblus asper*	4.13	85.87	20.79	6.62	> 100	> 15.105
3	Nyctaginaceae	*Boerhavia repens*	9.63	98.99	10.28	7.58	> 100	> 13.192
4	Clerodendaceae	*Clerodendrum viscosum*	42.43	81.09	1.91	27.01	> 100	> 3.702
5	Acanthaceae	*Adhatoda vasica*	59.59	> 1000	> 16.78	291.87	> 1000	> 3.43
6	Amaranthacae	*Amaranthus spinosus*	64.14	> 1000	> 15.59	135.19	> 1000	> 7.39
7	Rutaceae	*Aegle marmelos*	ND	> 1000	ND	ND	> 1000	ND
8	Fabaceae	*Tamarindus indica*	ND	> 1000	ND	ND	> 1000	ND
9	Rubiaceae	*Mussaenda corymbosa*	57.09	> 1000	> 17.52	ND	> 1000	ND

IC_{50}: 50% inhibitory concentration, CC_{50}: 50% cytotoxic concentration, SI: CC_{50} of 293T cell/IC_{50} of *P. falciparum* 3D7 strain, ND: Not determined.

In vitro invasion inhibition

The growth inhibition assay serves as a standard method for evaluating the overall effectiveness of a drug in inhibiting parasite invasion, rupture, and/or development (29). We, therefore, explored the effect of the plant extracts on the invasion of erythrocytes by parasites. Synchronized schizonts were cultured with the plant extracts for 24 h, after which parasitemia was evaluated (Fig. 6). The administration of the plant extract resulted in a significant reduction in parasite numbers (Fig. 6). Most of the affected parasites displayed a ring formation, suggesting that the plant extract had no impact on parasite rupture or egress.

In vitro cell cytotoxicity

The viability of 293T cells was evaluated in the presence of the nine plant leaf extracts. The absorbance at 450 nm in wells containing sterilized distilled water was used as the control, facilitating the calculation of 100% cell viability. The cell viability in the presence of the ethanolic and methanolic leaf extracts of *Ficus hispida*, *Streblus asper*, and *Boerhavia repens* was > 80% at 10 µg/ml (Fig. 7a-b). At the concentration of 100 µg/ml, the cell viability in the presence of methanolic extracts of *Clerodendrum viscosum* was > 90%. (Fig. 7b). In the presence of the ethanolic and methanolic leaf extracts of *Adhatoda vasica* and *Tamarindus indica*, cell viability was > 80% at a concentration of 1,000 µg/ml (Fig. 7c-d). Cell viability in the presence of the ethanolic and methanolic leaf extracts of *Amaranthus spinosus*, *Aegle marmelos* and *Mussaenda corymbosa* was > 60–80% at a concentration of 1,000 µg/ml (Fig. 7c-d). The exception was the methanolic extract of *Mussaenda corymbosa*, which exhibited a cell viability of > 80% at a concentration of 1,000 µg/ml (Fig. 7d). We calculated the *P. falciparum* selectivity of these extracts and identified that all extracts exhibited a selectivity index > 10, except for the ethanolic extract of *Clerodendrum viscosum*, which had an SI < 10 (Table 2).

Discussion

In this investigation, we explored the antiplasmodial activity and cytotoxicity of nine traditional medicinal plant species, each belonging to distinct families in Bangladesh. *Plasmodium* parasites undergo a cyclic process involving erythrocyte invasion, development, and egress, ultimately leading to the lysis of erythrocytes and the onset of severe anemia in the host. Therefore, each phase of the blood-stage parasite's life cycle presents a promising target for anti-parasitic interventions due to its crucial role in infection, although most current antimalarial drugs inhibit parasite development within the erythrocytes (30, 31). The leaves of all the plant species were used to determine the extraction yield, which is a measure of solvent efficiency employed to extract specific components from the original material (32). In this study, we used two solvents, ethanol and methanol, for the extraction of the active molecules from the plant leaves. The results from the extraction of all the different plants revealed that almost all the methanolic extracts provided a higher yield than the corresponding ethanolic extracts. This discrepancy may arise from differences in the extraction process parameters, with chemical constituents being more soluble in methanol than in ethanol, thereby resulting in a higher yield for the methanolic extract compared to the ethanolic counterpart. However, the efficacy of the ethanolic extract was found to be much higher than that of the methanolic extracts. Hence, an effective extraction method stands as a crucial parameter for achieving both a high total yield of an extract and the effectiveness of the plant molecules contained therein.

Streblus asper has emerged as a noteworthy medicinal plant with a myriad of therapeutic benefits *in vitro* and *in vivo* (33). These include anti-cancer, antibacterial, anti-fungal, anti-diarrheal, anti-macrofilaricidal, anti-diabetic, anti-inflammatory, anti-aging, and neuroprotective effects (33–36). It has been reported that *Streblus asper* extract exhibits antimalarial properties against murine malaria (21). To our knowledge, there have been no studies on the human malaria activity of this medicinal plant. Leaves and aerial parts of the plant have been reported to contain lupeol and oleanolic acids (33, 36). Lupeol and its derivatives have been shown to possess antiplasmodial activity in previous studies (37). In addition, oleanolic acid has also

been shown to possess antiplasmodial activity (38). In our investigation, the ethanolic and methanolic leaf extracts of *Streblus asper* exhibited high *in vitro* antiplasmodial activity, with concentrations < 10 µg/mL against *Plasmodium falciparum* 3D7. Moreover, these extracts were not cytotoxic to 293T cells, as indicated by an SI > 10. These findings highlight the potential of this extract as a valuable resource for the exploration and isolation of novel anti-malarial compounds. To our knowledge, this is the first IC_{50} value determination of *Streblus asper* against a human malaria pathogen. The identification of traditionally used plant extracts with IC_{50} values below 15 µg/mL represents a crucial initial step in the search for novel anti-malarial drugs. This discovery opens avenues for diverse strategies in drug development, contributing to ongoing efforts to identify effective treatments (39)

Ficus hispida, known as dumur (Bengali), is a good source of ethnomedicine, useful for wound healing, anti-inflammatory, antinociceptive, sedative, antidiarrheal, antiulcer, antimicrobial, antioxidant, hepatoprotective, antineoplastic, and antidiabetic activities (40–42). In our study, the ethanolic and methanolic leaf extracts of *Ficus hispida* exhibited IC_{50} values of 9.31 and 15.58 µg/ml, respectively, against the *P. falciparum* 3D7 strain. Interestingly, previous studies on *Ficus thonningii* leaves from Brazzaville, Republic of Congo, reported IC_{50} values of 16.05, 18.85, and 35.22 µg/ml for methanolic, ethanolic, and aqueous extracts, respectively, against *P. falciparum* 3D7 strain, and 9.61, 11.57, and 83.28 µg/ml for methanolic, ethanolic, and aqueous extracts, respectively, against the Dd2 strain (43). Furthermore, the antiplasmodial activity of methanolic root extract of *Ficus elastica* from Cameroon was reported with an IC_{50} value of 9.5 µg/ml against the *P. falciparum* 3D7 strain (44). In a study investigating the antiplasmodial activity of hydro-alcoholic extracts of *Ficus bengalensis and Ficus carica* from Iran, IC_{50} values of > 200 µg/ml were obtained against both chloroquine-resistant (K1) and chloroquine-sensitive (CY27) strains (45). Taken together, these findings, indicate that *Ficus hispida* is a valuable source of human antimalarial compounds, warranting further investigation. Plants belonging to the genus *Ficus* are known to be rich sources of antimalarial compounds.

To the best of our knowledge, our study is the first to evaluate the *in vitro* antiplasmodial activity of ethanolic and methanolic leaf extracts of *Clerodendrum viscosum* and *Amaranthus spinosus*, revealing IC_{50} values of 42.43 µg/ml and 64.14 µg/ml for the ethanolic extracts and 27.01 µg/ml and 135.19 µg/ml for the methanolic extracts, respectively. Silver nanoparticles with *Clerodendrum viscosum* leaf extract were reported to show antiplasmodium activity (46); however, we observed a low selectivity index for *Clerodendrum viscosum*, suggesting a potential cytotoxic effect on the host. Previous reports have highlighted the potent anticancer properties of *Clerodendrum viscosum*, demonstrating inhibition of the cell cycle and induction of apoptosis (47). Consequently, the use of molecules derived from this plant should be approached with caution to avoid potential adverse effects. Extracts from *Amaranthus spinosus* red stems in Africa have shown suppressive activity against *Plasmodium berghei*, with an effective dose (ED_{50}) of 789.36 ± 7.19 mg/kg (48). In our study, the IC_{50} values for the ethanolic and methanolic leaf extracts of *Adhatoda vasica* were 59.59 and 291.87 µg/ml, respectively. Notably, a previous study reported an IC_{50} value of 11.1 µg/ml for the methanolic extract of *Adhatoda vasica* roots from India (49). This discrepancy may be due to differences in the parts of the plants used.

In our study, the ethanolic leaf extract of *Mussaenda corymbosa* demonstrated an IC_{50} value of 57.09 µg/ml against *P. falciparum* 3D7 strain, whereas, for the methanolic leaf extract, we could not determine an IC_{50} value. A previous study reported that ethanolic extracts of sepals, leaves, and stems from *Mussaenda erythrophylla* and *Mussaenda philippica* species in Thailand exhibit potent activity against the *P. falciparum* K1 strain, with IC_{50} values of 258.3, 3.7, and 29.6 µg/ml and 287.3, 47.0, and 90.6 µg/ml, respectively (50). This discrepancy may be due to the variations the species and geographic location of the plant. Our findings clearly demonstrate that the *Mussaenda* genera are good source of human antimalarial compounds, and that ethanol is the best solvent for *Mussaenda* Genera plant extraction. In our investigation, ethanolic and methanolic leaf extracts of *Boerhavia repens* yielded IC_{50} values of 9.63 and 7.58 µg/ml, respectively, against the *P. falciparum* 3D7 strain. *Boerhavia elegans* from Iran has been reported to exhibit antiplasmodial activity in hydro-alcoholic extracts, with IC_{50} values of 15.33 and 11.97 µg/ml, respectively, against both chloroquine-resistant (K1) and chloroquine-sensitive (CY27) strains (45). So, our findings confirm that *Boerhavia repens* is an excellent source of human antimalarial compounds. Our study also revealed low activity against the *P. falciparum* 3D7 strain parasite for *Aegle marmelos* and *Tamarindus indica*. However, previous studies have reported antiplasmodial activity in the methanol extract (IC_{50} = 7 µg/ml) of *Aegle marmelos* leaves from India and the aqueous extract of *Tamarindus indica* fruit from Togo in Africa (IC_{50} = 4.786 µg/ml) (51, 52). Our observed low activity could be attributed to variations in the active constituents, potentially influenced by seasonal or geographic factors. Additionally, the complex interactions among phytoconstituents might lead to a masking effect, potentially reducing the overall activity of the extract (39). Furthermore, this variation could arise from differences in materials, experimental procedures, and the specific parasite strain employed. Metabolic profiles of plants can vary depending on factors such as plant species, plant parts, extraction solvent, and geographic distribution, influencing the observed outcomes.

Conclusion

We observed that ethanolic leaf extracts of *Ficus hispida, Streblus asper*, and *Boerhavia repens* are highly active against *Plasmodium falciparum* 3D7, exhibiting very low IC_{50} values. The presence of such low IC_{50} values suggests the existence of active plant molecules in the crude extract. These active molecules warrant isolation and further study to understand their effects on malaria models. Based on our findings, it is likely that additional antimalarial plant molecules will be discovered, and their active compounds investigated as part of efforts to develop new antimalarial drugs.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

UQ, KK, and MTAA designed the research. UQ and KK performed the experiments. UQ and MTAA analyzed the data. UQ wrote the paper. UQ, KK, and MTAA revised the manuscript.

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Figures

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Fig. 1

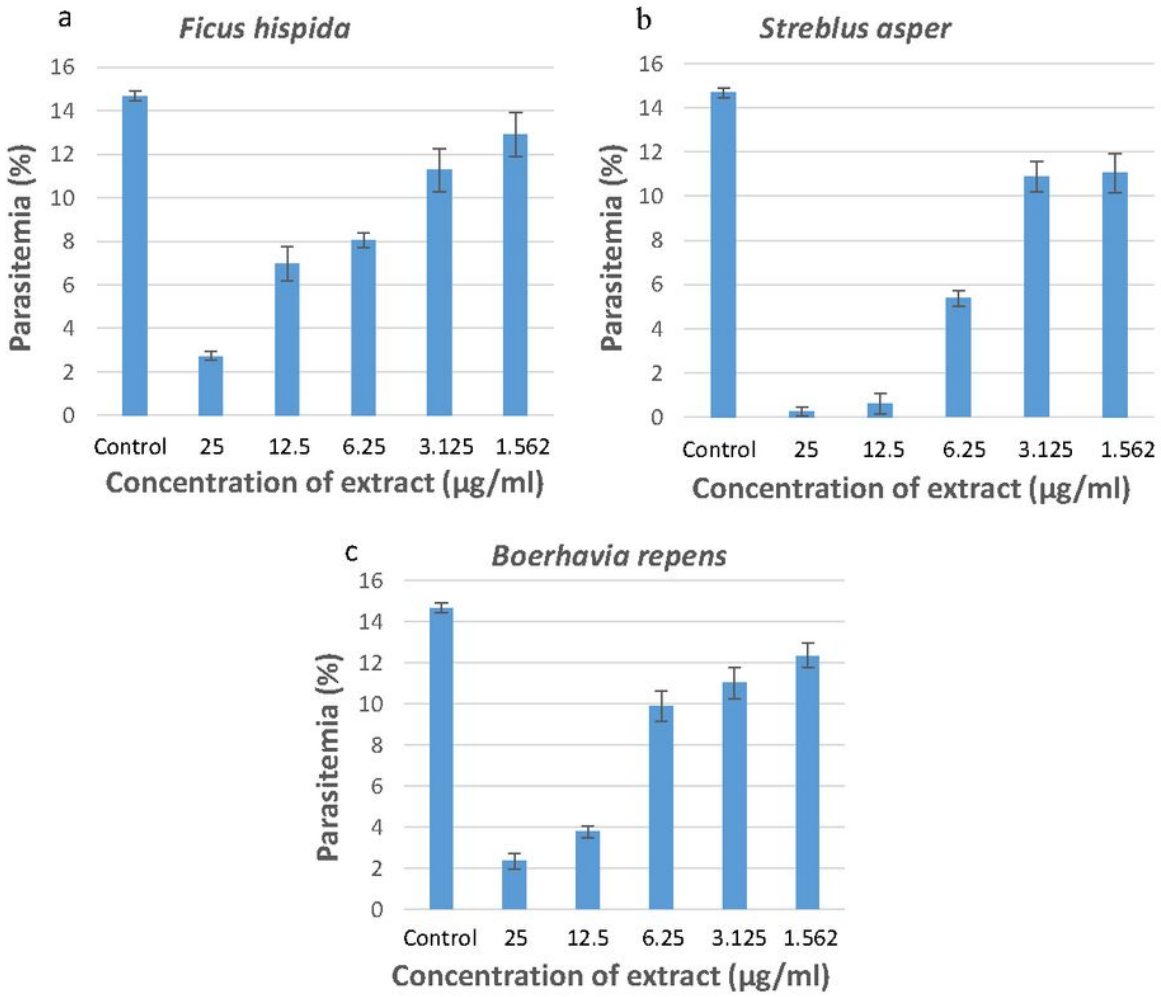

Figure 1

Ethanolic plant leaf extract antiplasmodial activity against *Plasmodium falciparum* 3D7. (a-c) Ethanolic extracts from *Ficus hispida*, *Streblus asper*, and *Boerhavia repens*were assessed for dose-dependent growth inhibition. Distilled water was used as a negative control. Various concentrations of each leaf extract were

prepared and incubated with *P. falciparum* 3D7 for 96 h. The X and Y axes represent the concentration of the extract (µg/ml) and the percentage of parasitemia, respectively. Error bars indicate the mean with standard deviation from three biological replicates of the experiments.

Fig. 2

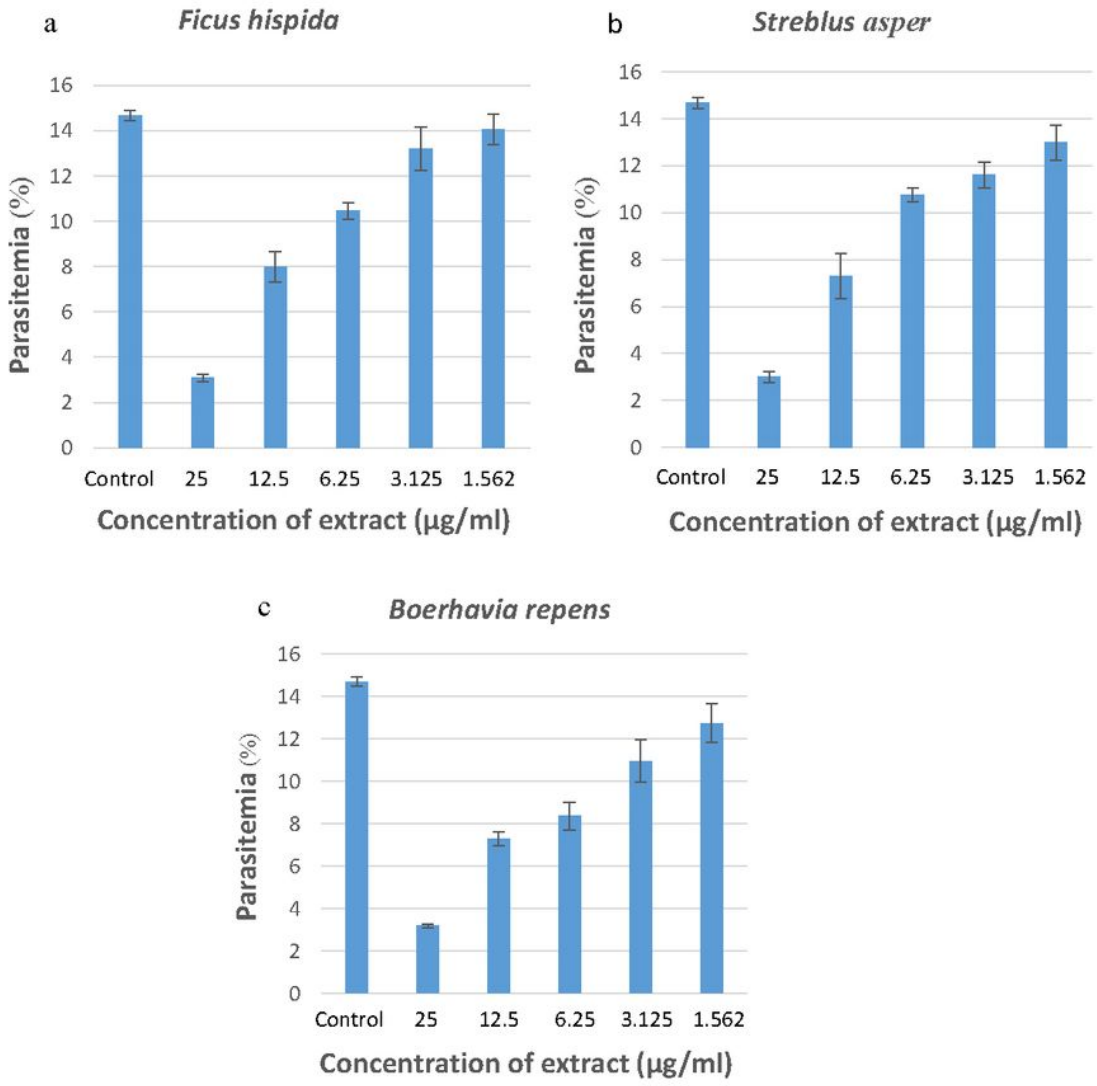

Figure 2

Methanolic plant leaf extract antiplasmodial activity against *Plasmodium falciparum* 3D7. (a-c) Methanolic extracts from *Ficus hispida*, *Streblus asper*, and *Boerhavia repens*were examined for dose-dependent

growth inhibition. Distilled water was used as a negative control. Different concentrations of each leaf extract were prepared and incubated with *P. falciparum* 3D7 for 96 h. The X and Y axes represent the concentration of the extract (µg/ml) and the percentage of parasitemia, respectively. Error bars indicate the mean with standard deviation from three biological replicates of the experiments.

Fig. 3 *Umme et al.*

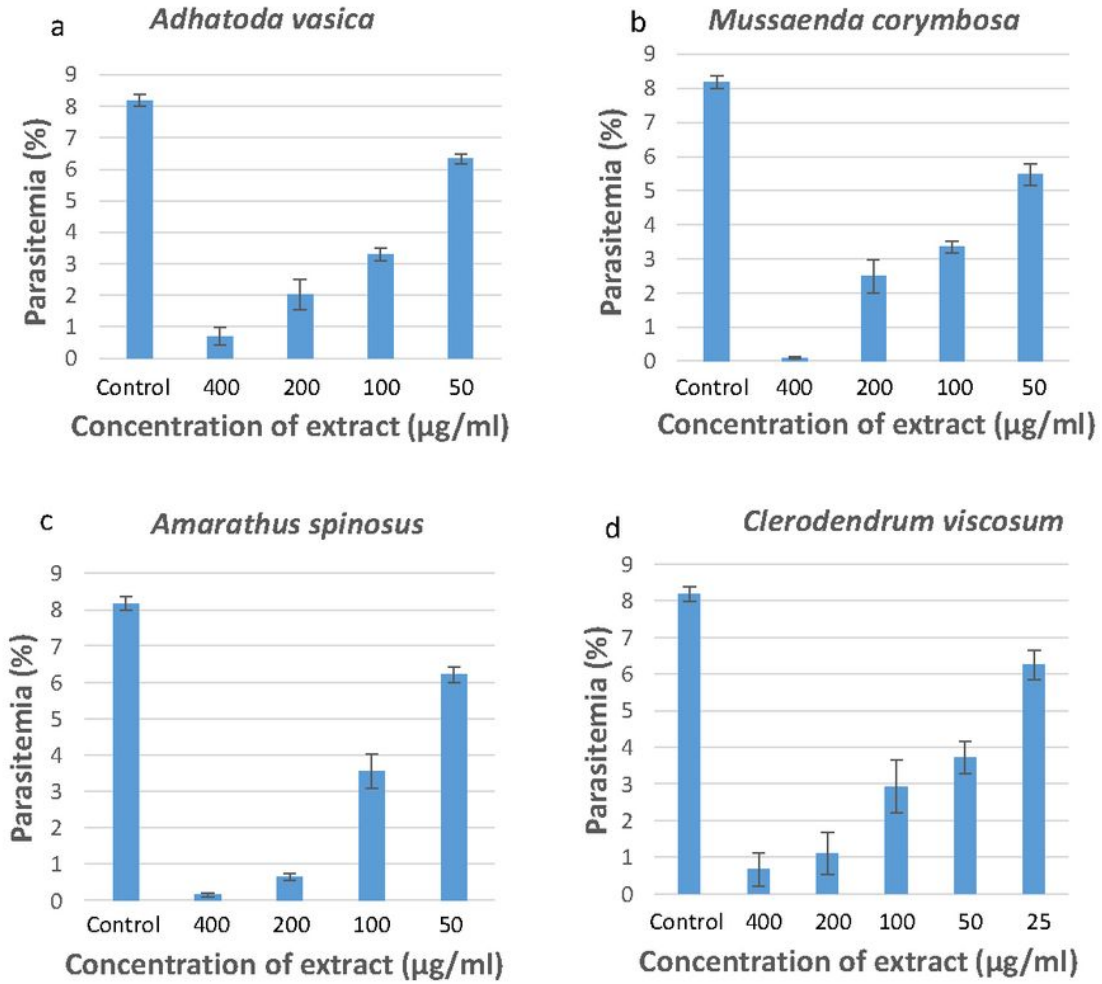

Figure 3

Antiplasmodial activity against *Plasmodium falciparum* 3D7 of additional ethanolic plant leaf extracts. (a-d) Ethanolic extracts from *Adhatoda vasica*, *Mussaenda corymbosa*, *Amaranthus spinosus*, and *Clerodendrum viscosum* were evaluated for dose-dependent growth inhibition. Distilled water was used as a negative control. Different concentrations of each leaf extract were prepared and incubated with *P. falciparum* 3D7 for 96 h. The X and Y axes represent the concentration of the extract (µg/ml) and the percentage of parasitemia, respectively. Error bars indicate the mean with standard deviation from three biological replicates of the experiments.

Fig. 4 *Umme et al.*

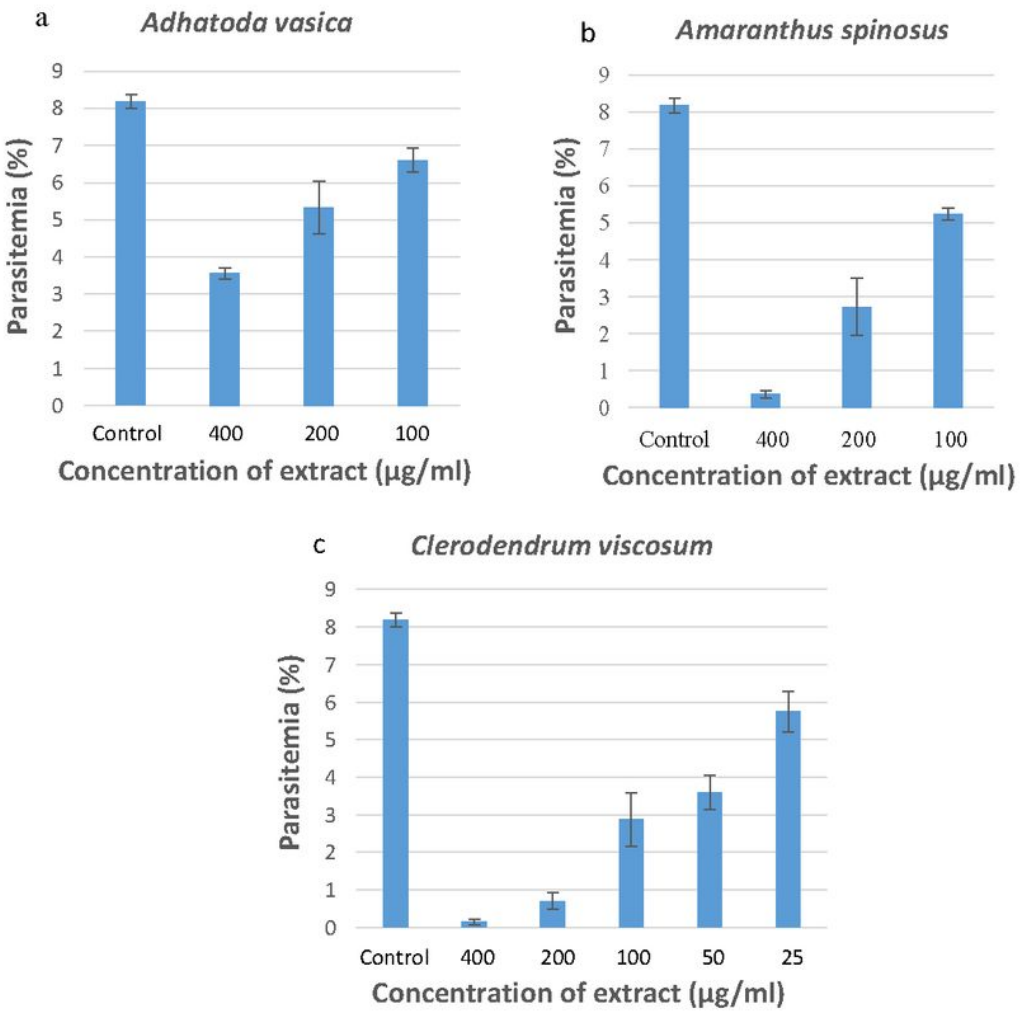

Figure 4

Antiplasmodial activity against *Plasmodium falciparum* 3D7 of additional methanolic plant leaf extracts. (a-c) Methanolic extracts from *Adhatoda vasica*, *Amaranthus spinosus*, and *Clerodendrum viscosum* were examined for dose-dependent growth inhibition. Distilled water was used as a negative control. Various concentrations of each leaf extract were prepared and incubated with *P. falciparum* 3D7 for 96 h. The X and Y axes represent the concentration of the extract (µg/ml) and the percentage of parasitemia, respectively. Error bars indicate the mean with standard deviation from three biological replicates of the experiments.

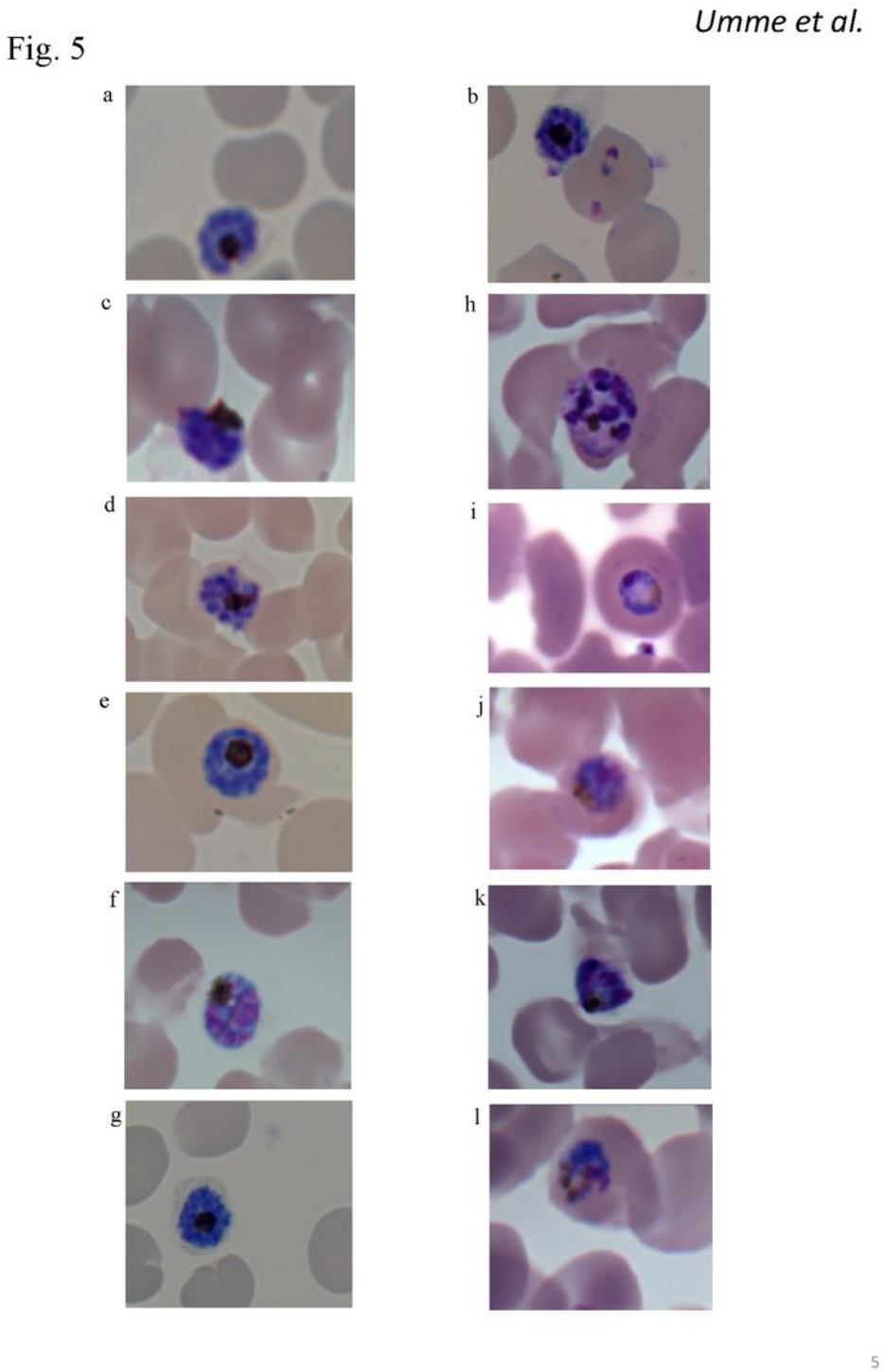

Figure 5

Microscopic observation of schizont-stage infected erythrocytes was conducted following the growth inhibition assay, post-treatment with the ethanolic and methanolic extracts. Ring-form infected erythrocytes were incubated for 96 h with (a) Artemisinin (ART) or (b) distilled water. ART served as a positive control; distilled water served as the negative control. Ethanolic extracts (c-g) and methanolic extracts (h-l) from *Adhatoda vasica*, *Amaranthus spinosus*, *Ficus hispida*, *Streblus asper*, and *Boerhavia repens*, respectively.

Fig. 6

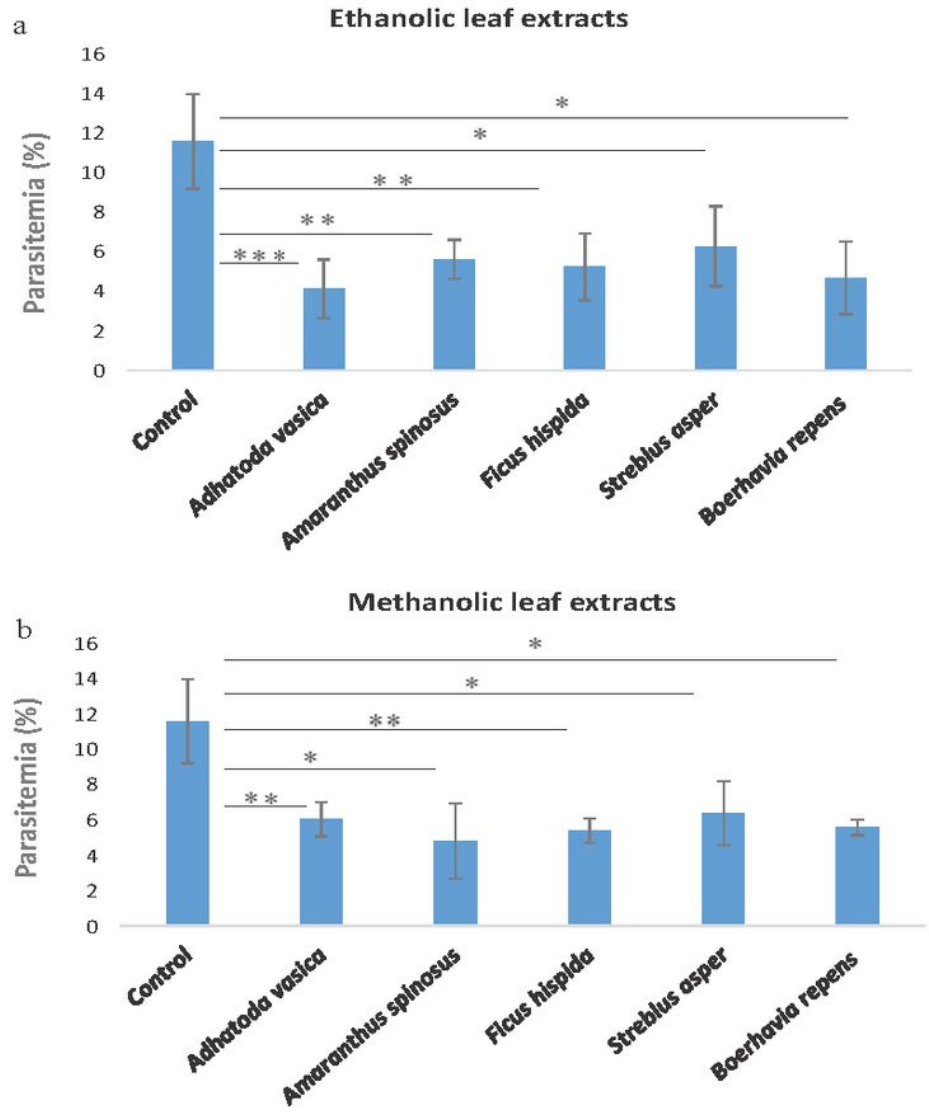

Figure 6

Statistical analysis of parasitemia reduction and inhibition of invasion by ethanolic and methanolic extracts from *Adhatoda vasica*, *Amaranthus spinosus*, *Ficus hispida*, *Streblus asper*, and *Boerhavia repens*. (a-b) Error bars indicate the mean with standard deviation from three biological replicates of the experiments. Statistical significance was determined by using a student t-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; compared with the negative control.

Fig. 7 *Umme et al.*

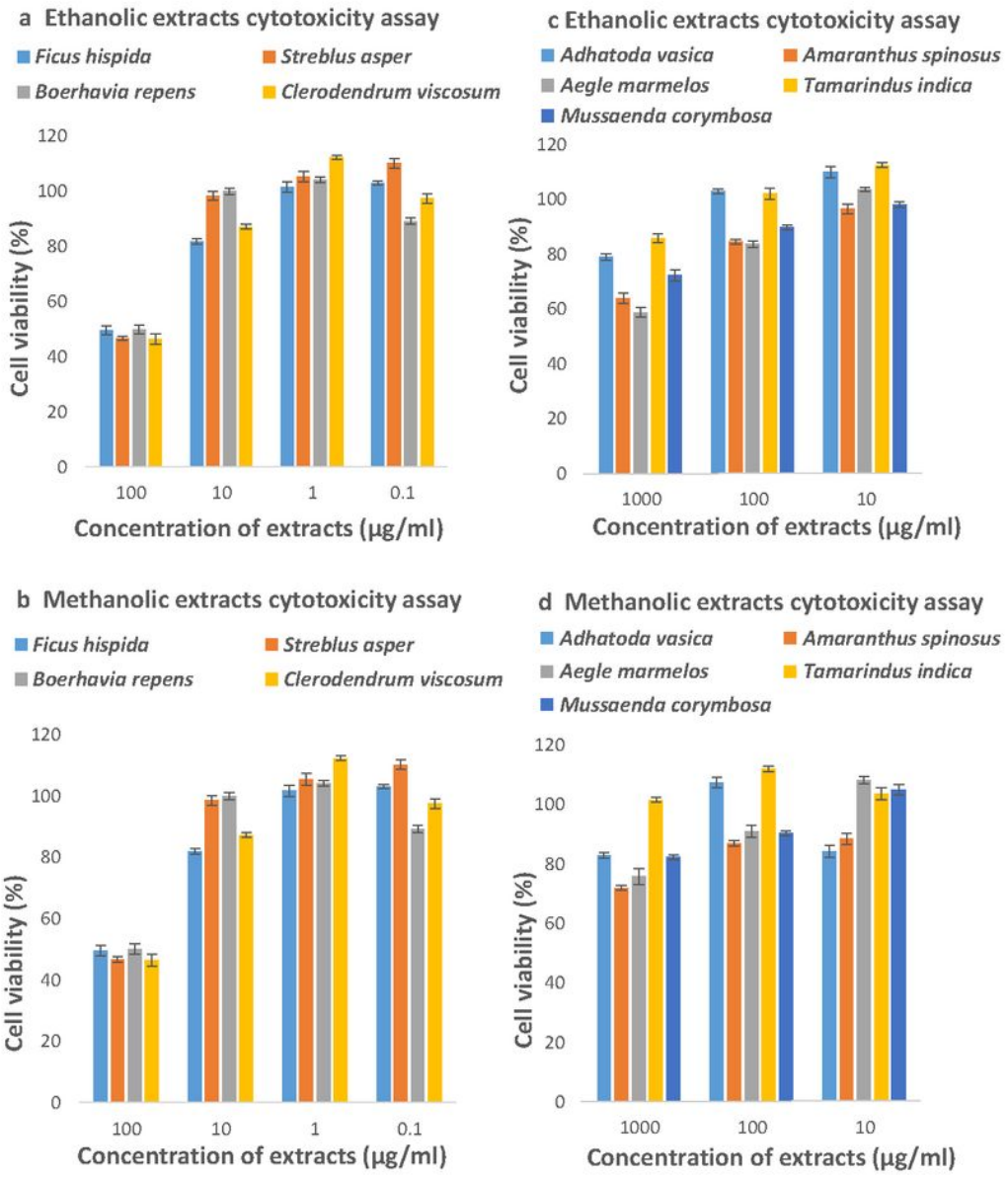

Figure 7

Cytotoxicity of ethanolic and methanolic plant leaf extracts to 293T cells. (a-b) Cytotoxicity assays were conducted with four anti-malarial extracts. The 293T cells were incubated with concentrations of 100, 10, 1, and 0.1 µg/ml of the test extracts for 96 h. High cell viability was observed at concentrations <10 µg/ml. (c-d) Cytotoxicity assays were performed using ethanolic and methanolic extracts from five anti-malarial plants. The 293T cells were incubated with concentrations of 1000, 100, 10, and 1 µg/ml of the extracts for 96 h. High cell viability was observed at concentrations <100 µg/ml.