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Gametocytocidal and Schizonticidal Activity of Methanolic and Hydroethanolic Extracts of *Entandrophragma angolense* (Welw.) C.DC. on the *in vitro* Maturation of Clinical Isolates of *Plasmodium falciparum*

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A B S T R A C T

Effective chemotherapy is an essential component in the fight against malaria. Thus, in the face of the emergence and rapid spread of resistance to artemisinin derivatives, a molecule used as first-line treatment for malaria, it is important to search for new antimalarial molecules. The eradication of malaria requires the research of antimalarial drugs with gametocytocidal effects. The objective of this work was to set up cultures propagating *Plasmodium falciparum* gametocytes from clinical isolates and to evaluate the gametocytocidal and schizonticidal activity of *Entandrophragma angolense* extracts. Gametocytes were produced with asexual forms of clinical isolates in culture. The gametocytocidal activity of the extracts was evaluated by microscopy. Schizonticidal activity was evaluated using the SYBR Green method. The formation of gametocytes until maturation was observed. Gametocytocidal activity was evaluated by the percentage of gametocyte inhibition. It ranged from 95.69% to 82.76% and from 93.1% to 63.83% respectively for the hydroethanolic and methanolic extract of *Entandrophragma angolense*. The inhibitory concentration 50 percent of the extracts was determined. It ranges from 05.73 to 14.76 µg/mL. Generally, the extracts showed significant gametocytocidal and schizonticidal activity. This work is a precursor for the research of gametocytocidal molecules, a source of antimalarial molecules blocking malaria transmission.

Introduction

Malaria is a disease caused by an intracellular parasite of the genus *Plasmodium*. *Plasmodium* life cycle requires gametocyte production and is transmitted to vectors from the human host. In 2020, malaria caused approximately 241 million cases and 627,000 deaths worldwide. Thus, WHO African region alone accounts for 95% of cases, or 228 million. The South-East Asia region accounted for nearly 2% of cases in 2020 (WHO, 2021). Children under 5 years, travelers from non-endemic areas, and pregnant women are particularly vulnerable (WHO, 2019). *Plasmodium falciparum* is considered to be the most lethal species (WHO, 2010). Today, the rapid spread of *Plasmodium falciparum* resistant to the usual antimalarials drugs such as chloroquine, and sulfadoxine-pyrimethamine (Ariey *et al.,* 2014; Dondorp *et al.,* 2009; Jambou *et al.,* 2005) and recently to artemisinin derivatives, molecules currently used as first-line drugs (Amaratunga *et al.,* 2016; Ashley *et al.,* 2014; Leang *et al.,* 2015) now pose a threat to malaria control and elimination and make the discovery of new antimalarial compounds urgent.

Gametocytes provide malaria transmission to the mosquito although they are not responsible for clinical symptoms. Most antimalarial drugs target the asexual forms of the parasite, which are responsible for the clinical symptoms of malaria. However, for transmission to occur, gametocytes must be transmitted from the human host to the Anopheles mosquito (Okell *et al.,* 2008). Gametocytes are less susceptible to all commonly used antimalarial drugs, with the exception of primaquine (Benoit-Vical *et al.*, 2007). Primaquine is currently the only approved antimalarial drug that is effective against *Plasmodium falciparum* gametocytes. But it has some drawbacks such as its propensity to cause acute hemolysis (Peatey *et al.*, 2012). Due to the emergence and spread of drug resistance and unsuccessful attempts to develop vaccines based on antigens from the asexual blood forms, it is important to focus on the gametocytes forms of the parasite to prevent transmission. Current efforts to eliminate malaria worldwide are progressing, and one approach to achieving this is the mass screening of medicinal plants for their potential gametocytocidal properties. The search for gametocytocidal antimalarial drugs has become important due to the global quest to eradicate the disease advocated by the WHO (WHO, 2015). Thus, the introduction of *in vitro* gametocyte production assays would be useful to evaluate new molecules and the efficacy of antimalarial drugs. The challenge today is to produce gametocytes *in vitro* culture from clinical isolates to test new molecules. It is in this perspective that this study was initiated to determine the effect of plant extracts on the development of gametocytes. The aim is to set up *in vitro* cultures propagating the sexual forms of *Plasmodium falciparum* obtained from clinical isolates and to evaluate the gametocytocidal and schizonticidal activity of hydroethanolic and methanolic extracts of *Entandrophragma angolense* bark.

Materials and Methods

Collection of plant material

In this study, the plant material consisted of *Entandrophragma angolense* bark harvested in August 2020 to Gomon in the Department of Sikensi (South city of Côte d'Ivoire). The collected samples were identified at the National Floristic Center (CNF) of the University Félix Houphouët-Boigny where a specimen is kept and registered under the number UCJ012284.

Preparation of plant extracts

The harvested plant material was dried at laboratory temperature for 3 to 4 weeks and then ground to a fine powder using a Retsch GM 300 grinder. The powder was used to prepare the different plant extracts. The extractions were performed according to the method of (Tuo *et al.,* 2015). Four (4) successive extractions by solvents of increasing polarity were performed in this order; hexane, ethyl acetate, ethanol, and methanol (Figure 1).

Preparation of media

A complete medium (CM) supplemented with hypoxanthine (CM + HX) was prepared. Thus, to 450 mL of RPMI 1640 medium (Eurobio) were added 12.5 mL of HEPES buffer (25 µM, Eurobio) and 5 mL L-glutamine (200 mM, Eurobio). Then, 50 mL of stock solution 1 (to 500 mL of RPMI 1640 is dissolved 25 g of albumax II and 10 g of glucose) and 625 µL of gentamicin (40 mg/mL, Eurobio) are added to the mixture to constitute the complete medium.

Then, to 450 mL of this medium, 50 mL of stock 2 medium is added (to 500 mL of RPMI 1640 is dissolved 2.5 g of hypoxanthine) to constitute the complete medium with hypoxanthine added. A volume of 200 mL of heparinized medium was also prepared at a concentration of 20 U/mL.

Collection of clinical isolates and preparation of parasitized red blood cells

After a clinical diagnosis of simple malaria, patients who came for consultation at the Urban and Community Health Unit of AboboAnonkoua-Kouté were referred to the laboratory for biological diagnosis by Rapid Diagnostic Test (RDT). Patients with a positive RDT for *Plasmodium falciparum* mono-infection underwent blood sampling. *Plasmodium falciparum* isolates were collected under sterile conditions in tubes containing EDTA from the elbow by venipuncture from individuals with uncomplicated malaria and sent to the Malariology laboratory of Institute Pasteur of Côte d'Ivoire.

The blood samples were anonymized and all remaining material was discarded to respect the confidentiality of the participants and the protection of personal data. Parasitized blood was washed three times with RPMI by centrifugation at 3000 rpm for 5 minutes to remove the plasma and white blood cells. At the same time, thick drops and blood smears were performed in duplicate to assess the parasitemia of the samples.

Production of gametocytes from *Plasmodium falciparum* clinical isolates

The methods of (Fivelman *et al.*, 2007) and (Beourou *et al.*, 2017; Duffy *et al.*, 2016) have been adopted for gametocyte production. Clinical isolates of *Plasmodium falciparum* at the trophozoite stage were maintained in culture between 0.5 and 2% parasitemia, and 5% hematocrit in a 25 cm^2 sterile cell culture flask (NunclonTM; Brand Products; Denmark) with culture medium (complete medium + Hypoxanthine). Then, the culture is gassed with a special gas mixture (90% nitrogen, 5% CO2, and 5% O2). The culture is then confined in a candle bell and placed in an incubator maintained at 37 °C. The culture medium is changed every day. Continuous monitoring of the culture is performed to follow the growth of the parasites. Ten (10) µL of the pellet was used to make a blood smear to evaluate the parasitemia of the culture.

Parasitemia was estimated by visual counting under a microscope (Olympus CX23; China) at the objective (x100) from a blood smear, stained with Giemsa (Giemsa's Reagent).

When the culture reached 3-5% ring forms, it was synchronized on a MACS magnetic column (LS Column; MiltenyiBiotec; GmbH; Germany), to concentrate these forms (Coronado *et al.*, 2013; Duffy *et al.*, 2016; Ribaut *et al.*, 2008; Spadafora *et al.*, 2011). Parasites were recultured at a parasitemia of 2.5% by diluting with fresh group O Rhesus-positive blood that was not parasitized. The next day, a blood smear is taken to check for aged or mature trophozoites. The following day, the presence of ring forms is verified by a blood smear. Then a double synchronization is done at seven (7) hours intervals on a MACS MultiStand magnetic column (MiltenyiBiotec; GmbH; Germany). If the parasitemia of the ring forms reaches 3 to 5%, the parasites are re-cultured to a parasitemia of 2.5% at a hematocrit of 8-10%. The Day After hematocrit is lowered to 2% by retaining the spent medium from the culture to stress the parasites. A volume of 20 mL of spent medium is replaced with fresh medium

over two (2) days. Over the next four (4) days, the medium is changed and heparin is added at a concentration of 20 U/mL according to (Miao *et al.,* 2013). Blood smears are taken, asexual forms decrease, and gametocytes are observed and peak about 2-4 days after the addition of heparin is stopped. Gametocyte parasitemia is determined by microscopic counting on 5000 red blood cells.

Evaluation of gametocytocidal activity

From a stock solution of concentration 1000 µg/mL, a concentration range by half dilution is prepared at 1000 µg/mL, 500 µg/mL, and 250 µg/mL.

In the wells containing 200 µL of this concentration range, 1800 µL of the inoculum was added to have a final volume of 2000 µL. Thus the concentrations were increased to 100 µg/mL, 50 µg/mL, and 25 µg/mL in each well respectively.

One well was reserved for the parasite growth control, without product, for this purpose, to 200 µL of complete medium, and 1800 µL of inoculum were added. The culture plates were confined in a candle bell and gassed with tri-gas consisting of 5% CO_2, 5% O_2, and 90% N_2 and placed in an incubator maintained at 37 °C. Testing was performed in 48-well culture plates for 72 hours. Blood smears were taken from the cultures every 24 hours for microscopic evaluation of gametocyte development and gametocyte count in 20 fields under immersion (Udeinya *et al.,* 2008). The gametocyte activity of the extracts was determined by calculating the percentage of parasite growth inhibition. The following formula is used for the calculation:

% I = 100 - (Test Parasitemia / Control Parasitemia)*100.

I: Percentage of parasite growth inhibition.

Evaluation of the schizonticidal activity

This test is based on the direct contact of the parasites with the active ingredient. Clinical isolates of *Plasmodium falciparum* were cultured in the presence of a concentration range of the studied extract during the *Plasmodium falciparum* life cycle. The concentration of extract inhibiting 50% (IC 50) of the parasite growth was determined. The tests were performed on 96-well microplates containing a volume of parasitized erythrocytes. Inhibition of erythrocyte schizonts (*Plasmodium falciparum*) is measured by the SYBR Green method (Akala *et al.,* 2011; Smilkstein *et al.,* 2004). Parasitemia during these tests was set between 0.3% and hematocrit at 2%. The tests were performed in duplicate microplates. The concentrations of extract that inhibit 50% of parasite growth (IC50) and the corresponding correlation coefficients were determined graphically, using WWARN's *In vitro*Analysis and Reporting Tool (IVART) software (Le Nagard *et al.,* 2011). Schizonticidal activity of plant extracts was classified according to (Bero and Quetin-Leclercq, 2011; Jansen *et al.,* 2012) and reported by (Beourou *et al.,* 2017) based on IC50s.

In vitro hemolytic property

The blood used to prepare the erythrocyte suspensions was collected in heparinized tubes for both tests (hemolytic and anti-hemolytic). After centrifugation of the blood at 3000 rpm for 5 minutes, the plasma was removed and the recovered pellet was washed 3 times with Phosphate Buffered Saline (PBS) (0.2 M, pH 7.4). After the last wash, the pellet is suspended again in PBS solution at a ratio of 1 volume of pellet and 9 volumes of PBS, achieving a 10% hematocrit (Haddouchi *et al.,* 2016; Rani *et al.,* 2014).

In vitro hemolysis test

The hemolytic power of the extracts is performed *in vitro*at two (2) concentrations (3200, 1600 µg/mL) on a suspension of human erythrocytes in PBS (Phosphate Buffered Saline). The hemolytic effect test of the extracts is performed according to the method of (Jansen *et al.,* 2012). To perform the hemolysis test, 30 µL of stock solution is placed in a hemolysis tube and mixed with 570 µL of 10% red

blood cells. Control solutions are also prepared. The negative control consisting of 30 µL of PBS + 570 µL of 10% red blood cells and the positive control consisting of 30 µL of 1% Triton X-100 + 570 µL of 10% red blood cells. Each sample was tested in triplicate (including control solutions) and incubated at 37°C for 60 minutes.

This experiment was repeated at least three times so that the experimental error would not exceed 10% (Guo-Xiang and Zai-Qun, 2008). After the incubation, these are gently mixed and put in an ice bath to stop the reaction. Finally, the tubes are centrifuged for 5 minutes at 3000 rpm and 200 µL of supernatant is transferred to a 96-well plate. The absorbance of the supernatant is read at 450 nm with a plate reader (Micro Plate Read, Elisa Plate Analyser, Gentaur). The following formula is used to calculate the percentage of hemolysis:

% hemolysis = [(A sample tested - A negative control)/(A positive control - A negative control)] x 100.

A: Absorbance at 450 nm.

Protective effect of the extract against osmotic stress associated with high temperature

The anti-hemolytic effect of plant extracts is evaluated *in vitro*using an erythrocyte model. The latter is easily isolated from blood and its membrane has similarities with other cell membranes (Shobana and Vidhya, 2016).

To test the anti-hemolytic effect of our extracts, assays using an erythrocyte model and then hemolysis induced by reduced salinity of the RBC suspension medium (hypotonic medium), combined with heat stress (elevated temperature) were developed.

Exposure of the erythrocyte to hypotonic stress combined with heat stress results in lysis of the erythrocyte membrane, accompanied by the release of hemoglobin. The following test assesses the protective effect of *Entandrophragma angolense* extracts on erythrocyte cells that have undergone these stresses. This test is performed according to the protocol of (Ganesh *et al.*, 2013). The percentage of hemolysis inhibition is calculated after reading the absorbance of the supernatant at 450 nm, using a spectrophotometer (Micro Plate Read, Elisa Plate Analyser, Gentaur) according to the following formula:

I% = 100- (Do sample/Do control) x 100

I %: Percentage of anti-hemolysis activity

Do sample: Absorbance of the sample

Do control: Absorbance of the control.

Results and Discussion

In vitro culture and induction of *Plasmodium falciparum* gametocytes

Clinical isolates of *Plasmodium falciparum* at the trophozoites stage are used to produce gametocytes. The different stages of gametocytes were observed from day 5 to day 13 of the culture after induction as shown in Figure 2.

Induction of gametocytogenesis is based on the fact that parasites could produce sexual forms by stress *in vitro* culture (Beourou *et al.*, 2017). Several factors are involved in the production of gametocytes *in vitro* namely; ages of culture, hematocrit, high parasitemia, and spent culture medium (Duffy *et al.*, 2016).

In this study, the maximum gametocyte density obtained was 1.23% with these clinical isolates *in vitro* culture, which is significantly lower than that obtained by (Beourou *et al.*, 2017), who obtained a gametocyte density of 1.26% with clinical isolates. In a 2013 study by (D'Alessandro *et al.*, 2013), the gametocyte density obtained was between 0.5% and 1.5% with the reference strain *Plasmodium falciparum* 3D7.

Gametocytes progress through a developmental pathway consisting of five morphological stages (I-V) until maturing at stage V (Duffy *et al.*, 2016; Hawking *et al.*, 1971). Figure 2 shows the different stages of gametocytes ranging from stage II to stage V. The production of gametocytes in culture would allow for molecular biology, biochemical, proteomic, and pharmacological studies. While the production of gametocytes in culture from laboratory clones is quite routine today, induction of *Plasmodium falciparum* field isolates into gametocytes at *in vitro* proliferation conditions remains uncertain and quite often unsuccessful (Gupta *et al.*, 2015).

Table.1 Gametocyte density of *Plasmodium falciparum* clinical isolates

Plasmodium falciparum isolate	Gametocyte density
ANK 21065	1.23%
ANK 21066	1.21%
ANK 21067	0.98%

ANK: Anonkoua-Kouté
The gametocyte density of the three isolates that were propagated into gametocytes varied from 0.98% to 1.23%.

Table.2 IC_{50} of different extracts on clinical isolates of *Plasmodium falciparum*

IC50 (µg/mL)			
		P7	**P9**
Clinical Isolates	**ANK21002**	7.46	06.25
	ANK21003	7.06	05.73
	ANK21004	11.72	09.80
	ANK21005	13.14	10.33
	ANK21006	12.36	14.76
	ANK21007	10.11	10.54

ANK: Anonkoua-kouté
P7: *Entandrophragma angolense* hydroethanolic extract
P9: Methanolic extract of *Entandrophragma angolense* bark

Table.3 *In vitro* hemolytic activity of *Entandrophragma angolense* extracts

Extracts	**Concentration (µg/mL)**	**Hemolytic activity (%)**
P7	3200	0.42± 0.00%
	1600	0.02 ± 0.00%
P9	3200	0.43 ± 0.00%
	1600	0.05 ± 0.01%
AS	3200	1.37 ± 0.03%
	1600	0.04 ± 0.00%
Negative control (PBS)	-	0± 0.00%
Positive control (Triton X-100 à 1%)	-	100 ± 0.02%

Fig.1 Preparation flow chart of hexanic, acetate-ethyl, hydroethanolic, and methanolic extracts. The extracts were coded as follows:
P7:Hydroethanolic extract of *Entandrophragmaangolense* bark
P9: Methanolic extract of *Entandrophragmaangolense* bark

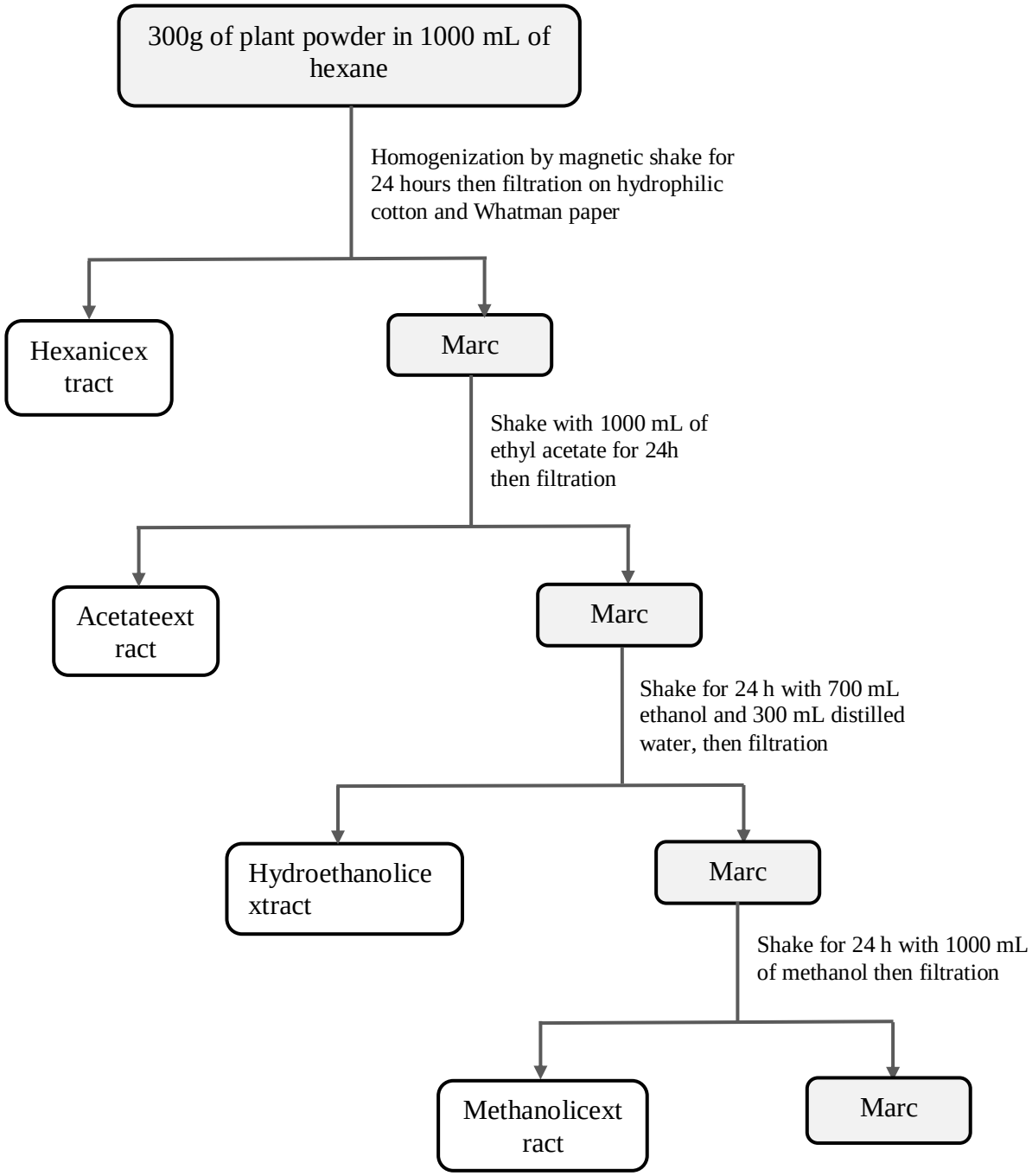
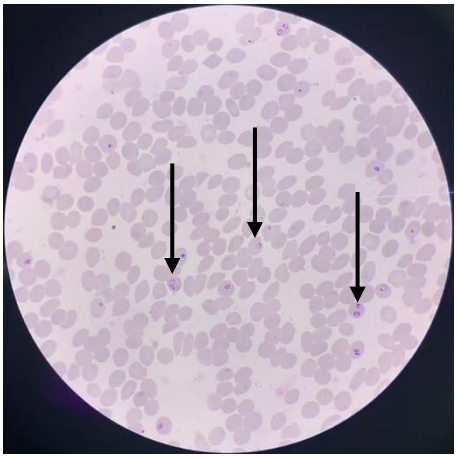
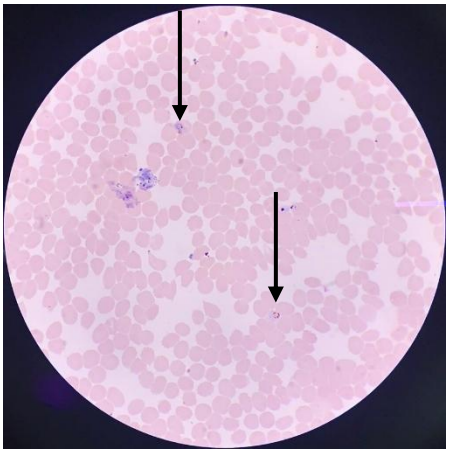


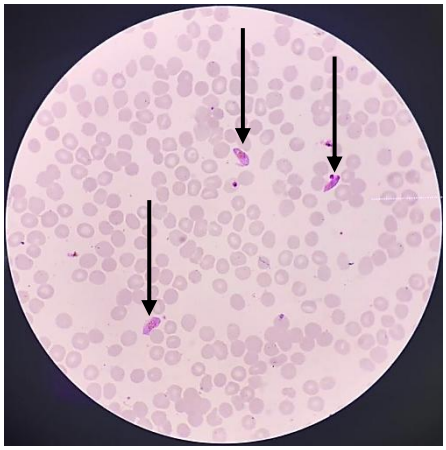
Fig.2 Different gametocyte stages of *Plasmodium falciparum*



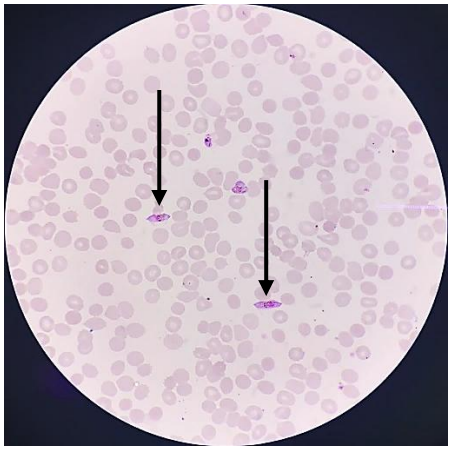
Trophozoite stages



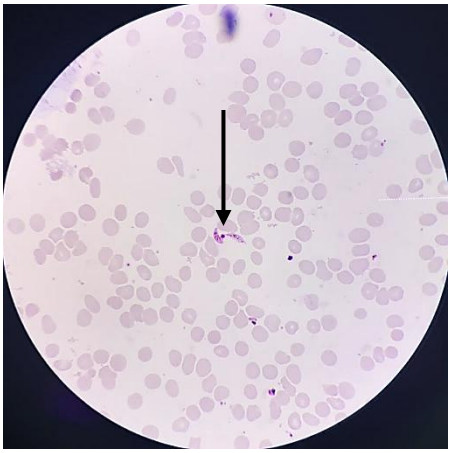
Stage I: days 5 à 6



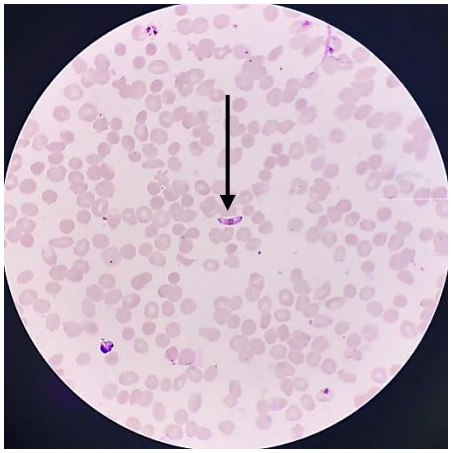
Stage II: days 7 à 8



Stage III: days 9 à 10



Stage IV: days 11 à 12



Stage V: days 13 à 15

The different gametocyte stages were observed as follows: Stage II: 7th - 8th day; Stage III: 9th - 10th day; Stage IV: 11th - 12th day; Stage V: 13th - 15th day after induction.

Fig.3 Gametocycidal activity of *Entandrophragma angolense* extracts
P7: Hydroethanolic extract of *Entandrophragma angolense* bark
P9: Methanolic extract of *Entandrophragma angolense* bark

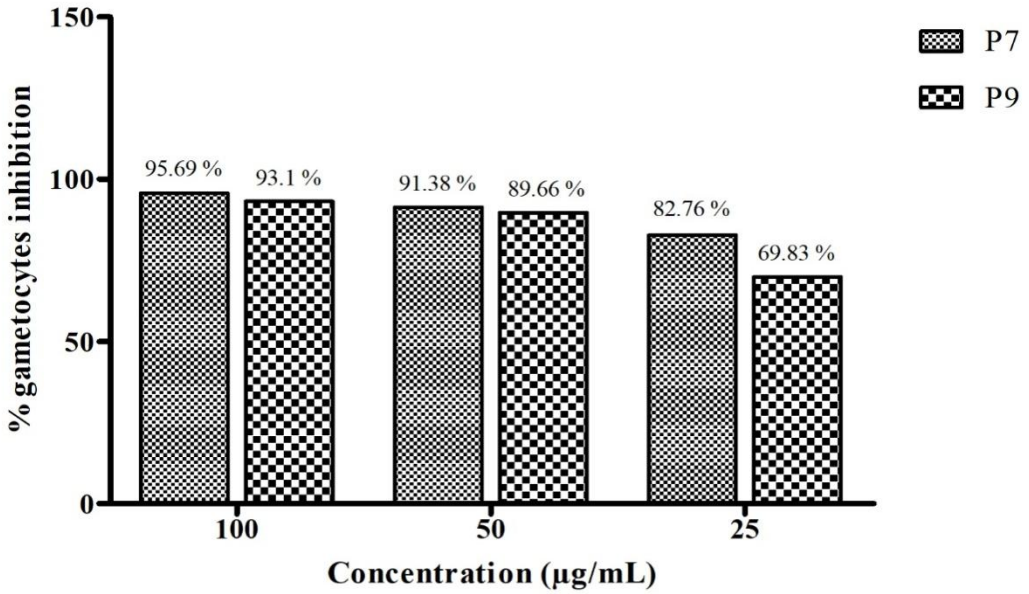
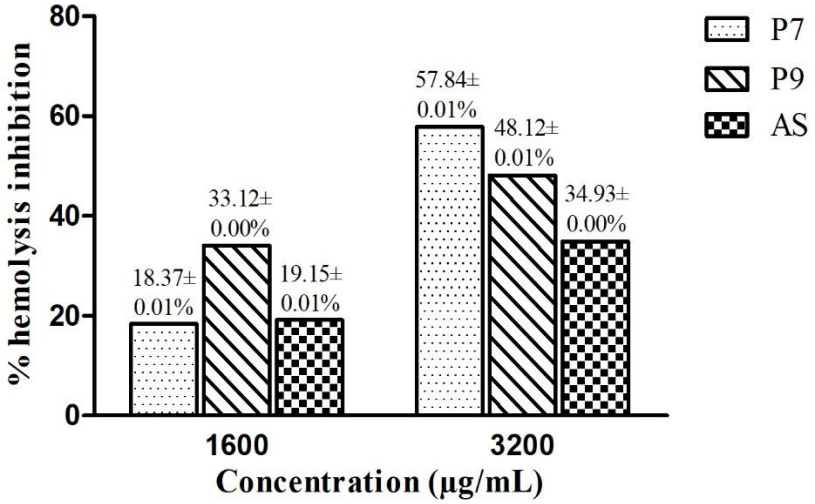


Fig.4 *In vitro* anti-hemolytic activity of *Entandrophragma angolense* hydroethanolic and methanolic extracts
P7: hydroethanolic extract *Entandrophragma angolense* bark
P9: Methanolic extract of *Entandrophragma angolense* bark
AS: Aspirin



Gametocytocidal activity of *Entandrophragma angolense* hydroethanolic and methanolic extracts

Gametocytocidal activity of the hydroethanolic and methanolic extracts of *Entandrophragma angolense* was evaluated at three concentrations 100 µg/mL; 50 µg/mL and 25 µg/mL. The percentage of gametocyte inhibition was for each extract concentration (Figure 3). The percentage of gametocyte inhibition ranged from 95.69% to 69.83%. The hydroethanolic extract (P7) showed potent gametocyte inhibition with a percentage inhibition of 95.69%. The lowest percentage of inhibition was observed with the methanolic extract (P9) with a percentage of 69.83%.

Gametocytocidal effect of antimalarial drugs has become important in malaria research due to the global quest to eradicate the disease. With the new global agenda on total malaria eradication, local antimalarial drugs need to be tested to see if they actually block parasite transmission. In this study, the gametocytocidal activity of the hydroethanolic and methanolic extracts of *Entandrophragma angolense* bark was evaluated (Figure 3). The hydroethanolic extract showed significant activity with a percentage of gametocyte inhibition of 95.69% at the concentration of 100 µg/mL while the methanolic extract obtained a percentage of gametocyte inhibition of 93.10% at this same concentration. At the lowest concentration of the extracts, the percentage of inhibition was 82.76% and 69.83% respectively for the hydroethanolic extract and the methanolic extract. Previous works have also demonstrated the gametocytocidal activity of some molecules on *Plasmodium falciparum* 3D7 gametocytes.

The activity of dihydroartemisinin, epoxomicin, and primaquine was evaluated on *Plasmodium falciparum* 3D7 gametocytes. The inhibitory concentrations 50 (IC 50) obtained were 17.0 ± 7.3 nM, 3.9 ± 0.8 nM, and 12515 ± 6541 nM respectively for dihydroartemisinin, epoxomicin, and primaquine after 72 + 72 hours of incubation (D'Alessandro *et al.*, 2013). Elimination and eradication of malaria can be achieved if the transmission cycle of the parasite is interrupted. These extracts have shown very strong gametocytocidal activities.

Schizonticidal activity of *Entandrophragma angolense* hydroethanolic and methanolic extracts

The use of *exvivo* culture of *Plasmodium falciparum* is one of the means used today in the search for molecules with antiplasmodial activity (Eze *et al.*, 2013; Karamoko *et al.*, 2019). The *exvivo* inhibitory activity of hydroethanolic and methanolic extracts of *Entandrophragma angolense* barks was evaluated on field isolates of *Plasmodium falciparum*. Table II shows the results of the schizonticidal activity of the tested extracts on field strains of *Plasmodium falciparum*.

Extracts of hydroethanolic and methanolic obtained IC50 less than 15 µg/mL. These extracts showed promising schizonticidal activity. The most promising schizonticidal activity was observed by the methanolic extract with an IC50 of 05.73 µg/mL on the field strain ANK21003. This interesting antiplasmodial activity obtained from *Entandrophragma angolense* extracts has been demonstrated in other studies (Beourou *et al.*, 2017; Bickii *et al.*, 2007b, 2007a).

Hemolytic activity

The hemolytic activity of the extracts was studied against human erythrocytes. The results of the cytotoxicity test presented percentages of hemolysis of red blood cells, depending on the concentrations of different extracts. These results are shown in Table III.

Hemolytic activity provides primary information about the interaction between molecules and biological entities at the cellular level. The hemolytic activity of any compound is an indicator of general cytotoxicity toward normal healthy cells

(Tuo *et al.,* 2022). The results obtained in this study indicated the absence of hemolytic activity. The results showed less than 1% hemolytic activity for *Entandrophragma angolense* extracts at all extract concentrations tested. The highest hemolytic activity was observed with aspirin, used as a reference molecule. It is equal to 1.37% at the concentration of 3200 µg/mL. The extracts of *Entandrophragma angolense* show a very low toxic effect against isolated erythrocytes. A hemolytic potency is defined if the percentage of hemolysis is greater than 5% (Jansen *et al.,* 2012; Laurencin *et al.,* 2012).

Protective effect of extracts against hemolysis induced by osmotic stress associated with high temperature

Hemolytic inhibition is observed by adding extracts of *Entandrophragma angolense* to red blood cells subjected to osmotic shock associated with elevated temperature to induce hemolysis. *Entandrophragma angolense* extracts at concentrations of 1600 and 3200 µg/mL protected the lysis of the human erythrocyte membrane. Indeed, the interaction between the secondary metabolites of the extracts with erythrocyte proteins would probably be the cause of the protection against hemolysis.

Injury to the red blood cell membrane makes the cell vulnerable to free radical damage (Chakraborty and Shah, 2011). The ability of plant extracts to prevent red blood cell hemolysis was demonstrated in this study. *Entandrophragma angolense* extracts were found to inhibit hemolysis caused by osmotic stress associated with elevated temperature. The maximum inhibition of 57.84 ± 0.01% was observed at the concentration of 3200 µg/mL of *Entandrophragma angolense* hydroethanolic extract (Figure 4). Hemolysis inhibition studies were performed with extracts of *Mimusops elengi* (Ganesh *et al.,* 2013) and *Annona muricata* (Muthu and Durairaj, 2015). The reference (Sravani *et al.,* 2015), showed that extracts of *Mangifera indica* and *Manilkarazapota* also had anti-hemolytic activity. Based on these obtained results, the extracts of *Entandrophragma angolense* were likely to stabilize the membrane of red blood cells, hence their anti-hemolytic properties.

In vitro culture propagation of gametocytes is important in the global quest to eliminate and eradicate malaria. Gametocyte propagation of naive clinical isolates was successful in this study. Chemosensitivity tests on sexual and asexual forms of *Plasmodium falciparum* clinical isolates were demonstrated. It revealed that hydroethanolic and methanolic extracts of *Entandrophragma angolense* bark were active on asexual forms as well as gametocytes of cultured *Plasmodium falciparum* clinical isolates. Such drug effects reduce the transmission of the parasite among its hosts.

The active compounds in these extracts can therefore be studied as malaria transmission-blocking agents. These extracts could be a natural source of antimalarial molecules. The present study also showed that the extracts of *Entandrophragma angolense* stabilized the membrane of red blood cells against heat-induced hypo-tonicity and membrane lysis.

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